ANALYSIS OF PERMEABLE BARRIER TECHNOLOGY AS AN IN SITU GROUNDWATER REMEDIATION TOOL USING POLYVINYL ALCOHOL IMMOBILIZED CELLS

By

PATTY S. THOMPSON

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1974

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1988

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1996

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Thesis Approved:

Thesis Adviser

Gregory J. Wilba

William F. McTernan

Thomas C. Collins

Dean of the Graduate College

ACKNOWLEDGMENTS

My deepest appreciation goes to my husband, Patrick, and my sons, Charles and Grant who provided persistent encouragement, and to whom this thesis and author are dedicated. A special thanks is given to John Lee for his encouragement and support.

I want to express my appreciation to Fatemeh Razavi Shirazi, my coconspirator, for her help and friendship.

I extend my sincere thanks to Dr. John Veenstra, my principal advisor, for his professional guidance and assistance during this project. I also wish to thank Dr. Greg Wilber and Dr. William F. McTernan for their support during my graduate studies at Oklahoma State University and for being on my advisory committee.

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LIST OF NOMENCLATURE

BOD	biochemical oxygen demand
COD	chemical oxygen demand
CP	chlorophenol(s)
DAPI	4,6-diamidino-2-phenylindole
DCP	dichlorophenol
DO	dissolved oxygen
ECD	electron capture detector
FID	flame ionization detector
GC	gas chromatograph
IC	ion chromatograph
ICl	inorganic chloride
LAM	linear adsorption model
M	molarity
MCP	monochlorophenol
MS	mass spectrometry
MW	molecular weight
N	normality
PAC	powdered activated carbon

PCP pentachlorophenol

PVA polyvinyl alcohol

r_g growth rate

r_{su} substrate utilization rate

SCM shrinking core model

SEM scanning electron microscope

TBP tribromophenol

TCP trichlorophenol

TeCP tetrachlorophenol

T-N total nitrogen

TOC total organic carbon

VSS volatile suspended solids

CHAPTER I

INTRODUCTION

Background

Modern industry made use of many chemicals without knowing their full impact to the environment until the latter part of this century. Groundwater polluted by these chemicals is now recognized as a major environmental problem because approximately 50% of the United States population depends upon groundwater as its primary source of drinking water (Pye et al., 1983). Among these chemicals are chlorophenols. Chlorophenols are toxic aromatic organic compounds that are mainly used as biocides and fungicides in wood preservation (WHO, 1989). Chlorophenols are released into the environment from production sites, and are also introduced into the environment as unintentional by-products from industrial and municipal chlorination processes (WHO, 1989). The United States Environmental Protection Agency (USEPA) included chlorophenols on its priority list of the most common hazardous substances found in the United States (52 FR 12866, April 17, 1987; 53 FR 41279, October 20, 1988) and mandated a maximum contaminant level (MCL) in drinking water of 0.1 μg/liter for pentachlorophenol (40 CFR §141.61). Chlorophenols are toxic, have low taste and odor thresholds, bioaccumulate, and tend to be persistent in the environment (USEPA, 1980c).

Elevated concentrations of chlorophenols in groundwater are directly attributable to industrial pollution caused from spills and leaching. Isolated levels as high as 100 mg/L of chlorophenols in groundwater have been reported (Valo et al., 1990). Therefore, contamination of groundwater by chlorophenols is of concern and remediation is warranted.

New and effective groundwater remediation technologies are needed to remove or biodegrade chlorophenols. There are two basic objectives in groundwater remediation: (1) containment and (2) removal (Rael et al., 1995). Some of the more conventional remediation technologies being used singularly or in combination to meet these objectives are pump and treat, soil vapor extraction, air sparging, air stripping, granulated activated carbon, slurry wall containment, and in situ bioremediation (Olsen and Kavanaugh, 1993).

In situ permeable barriers are a relatively new "cost-effective" technology that can be used in groundwater remediation of shallow aquifers (Thompson et al., 1991). The barrier medium would allow the flow of water, but would sorb or react with the contaminant, preventing further migration. Crushed limestone, peat, and powdered activated carbon are several effective barrier mediums that have been used to adsorb or precipitate contaminants (Rael et al., 1995). This study proposes to examine the feasibility of using polyvinyl alcohol beads containing immobilized bacteria as an alternative permeable barrier media that would create a "bio-trench" or "bio-curtain" to biodegrade chlorophenols in situ from groundwater. The "bio-trench" concept (Figure 1) consists of a permeable barrier of PVA-immobilized cells placed in a trench. The

PVA-immobilized cells create a porous and permeable barrier designed to biodegrade contaminants as groundwater flows through the medium.

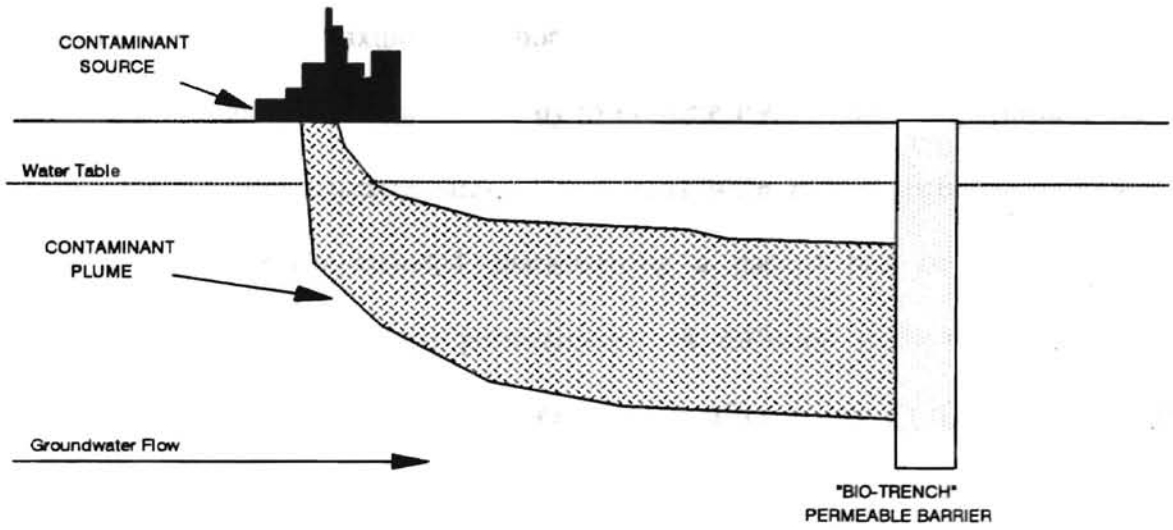


Figure 1. Bio-trench concept.

Immobilized cells are an established technique used in the wastewater treatment field that could be applicable to in situ groundwater treatment (Yang et al., 1989; Stormo and Crawford, 1994). One of the more widely used techniques for cell immobilization is cell entrapment. Microorganisms are enclosed within a porous polymeric matrix which allows the diffusion of substrate to and products from the entrapped microorganisms (Wu and Wisecarver, 1992). This technique has been recognized as a promising method for the biological removal of chlorophenols which are known to be recalcitrant (Sofer et al., 1990). Bettmann and Rehm (1984) determined that entrapped microorganisms were better protected against toxic chemicals than free cells. Sofer et al. (1990) further

demonstrated that entrapped microorganisms could withstand higher concentrations of toxic organic compounds than free cells.

This study consisted of immobilizing microorganisms in polyvinyl alcohol (PVA) and then dropping the mixture into a boric acid solution to form 3-5 mm diameter beads. Since this was an initial feasibility study to evaluate PVA beads as a permeable barrier media the physical characteristics of the beads were needed essential information. Therefore, this study included characterizing a packed bed of beads as to its compressibility, porosity, and permeability. A diffusion study was conducted to determine the rate of 2,4,6-trichlorophenol (TCP) diffusion into PVA beads. The batch study included placing various masses of blank beads in groundwater spiked with a constant concentration of TCP for the purpose of developing an isotherm to determine adsorption. A kinetic study was conducted to determine the substrate utilization rate of the mixed bioculture as free cells and as immobilized cells. In the initial column study, groundwater spiked with a constant concentration of 10.0 mg/L of TCP was fed into a column with a packed bed of PVA beads containing immobilized bacteria between layers of aquifer sand. The purpose of this column study was to simulate a "bio-trench" and determine the biodegradation rate of TCP by the continuous flow reactor. This study continued for 45 days. In the final column study, groundwater spiked with a constant concentration of 10.0 mg/L of TCP was fed into two columns of varied sizes with packed beds of PVA-immobilized cells. The purpose of this study was to compare different hydraulic retention times and removal efficiencies. This column study was monitored for 14 days.

Objectives

Chlorophenols are toxic organic compounds that have been found in various groundwater supplies. They pose a serious threat to the environment and warrant remediation. This study investigated an innovative method to biodegrade TCP in situ. The proposed method included using PVA-immobilized cells as an alternative permeable barrier media that would create a "bio-trench" to biodegrade chlorophenols in situ from groundwater. The objective of this feasibility study was to evaluate permeable barrier technology as an in situ groundwater remediation tool using PVA-immobilized cells by (1) studying the physical characteristics of PVA beads as a permeable barrier media; and, (2) studying the ability of this biological carrier system to biodegrade TCP.

CHAPTER II

LITERATURE REVIEW

Introduction

A background of current research is helpful in evaluating permeable barrier technology as an in situ remediation tool using PVA-immobilized cells as a medium designed to biodegrade TCP from groundwater. Physical and chemical characteristics of TCP are presented to give a better understanding as to its toxicity, its ability to degrade, and its effect on the environment. Different approaches to remove chlorophenols from groundwater have been evaluated as to their effectiveness, including biodegradation. Various methods and matrixes used to immobilize cells are described. Several different diffusion models are reviewed for their applicability in determining diffusivity coefficients. Finally, permeable barriers are reviewed as to their applicability for in situ remediation along with a comparison of different mediums used.

Chlorophenols

Chlorophenols (CPs) are organic chemicals formed from phenol by the substitution of one or more atoms of chlorine on the phenol ring. Nineteen congeners are possible (WHO, 1989). The following are some congeners formed from the direct

chlorination of phenol and are found polluting groundwater (WHO, 1989): 2-monochlorophenol (2-MCP), 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP). The compound TCP was concentrated on in this study because it was the highest chlorinated phenol available at the time this study began. TCP was determined to be carcinogenic to rats and mice (NCI, 1979; USEPA, 1980c) and human exposure should be kept to a minimum (WHO, 1989).

Pure chlorophenols are solid, colorless crystals at room temperature, except for 2-MCP which is a liquid (WHO, 1989; USEPA, 1980c). CAS numbers, common names, abbreviations, molecular formulas, and common synonyms and trade names for MCP, DCP, TCP, TeCP, and PCP are listed in Table 6, Appendix A. Chlorophenols have strong pungent odors and their taste and odor thresholds are so low that acceptable concentrations for drinking water are based on organoleptic rather than toxicological criteria (WHO, 1989). Hoak (1957) reported that the odor threshold increases as chlorination increases (e.g., from 0.33 μg/L for DCP to 12,000 μg/L for PCP). Solubility varies from 2.1 X 10^{-1} mole/liter for 2-MCP to 7.9 X 10^{-4} mole/liter for 2,3,4,6-TeCP (USEPA, 1980c). WHO (1989) reported that acidity increases in chlorophenols as chlorination increases. pH is an important factor in chlorophenol adsorption onto soils and they are inversely related. Volatility decreases as chlorination increases. The n-octanol-water partition coefficient increases with chlorination, which indicates a tendency for higher chlorophenols to bioaccumulate. Physical and chemical properties are identified in Table 7, Appendix A.

Large quantities of chlorophenols are used in wood preservation as biocides, fungicides and mold inhibitors and in lesser amounts in the production of antiseptics and disinfectants. They are also used as intermediates in the production of herbicides, dyes and drugs (WHO, 1989; USEPA, 1980c; USEPA, 1980).

Chlorophenols are released into the environment from production sites, during transportation, incineration, industrial wastes from wood preservation sites, saw mills, pulp and paper mills. Chlorophenols are generated unintentionally as by-products from municipal and industrial chlorination processes (WHO, 1989; USEPA, 1980c).

Chlorophenols strongly adsorb onto acidic and/or organic soils, but minimal adsorption occurs in alkaline conditions (WHO, 1989). Therefore, chlorophenols leach into the groundwater from soils that are alkaline or have low organic content or through material that can percolate readily (WHO, 1989).

Methods to Remove Chlorophenols from Groundwater

Litchfield et al. (1994) used a biotreatment-train approach for in situ bioremediation of a pentachlorophenol (PCP)-contaminated site. Recovered groundwater that had been pumped to the surface was passed through an ultraviolet light/ozone system and then enhanced with nutrients (including nitrate). The treated groundwater was gravity fed to seepage beds. A 90% reduction of PCP was achieved. After 2 years a fluidized bed-activated carbon tower unit replaced the ultraviolet/ozone system. PCP reduction averaged 93.1%. They concluded that not only was the PCP removed by the ultraviolet system and the activated carbon tower, but that additional removal by

biodegradation resulted from the stimulation of indigenous microorganism by the nutrients.

Biodegradation is an alternative remediation method that has successfully removed chlorophenols from groundwater (O'Reilly and Crawford, 1989; Järvinen et al., 1994; Pitter, 1976; Häggblom et al., 1988). Järvinen and Puhakka (1994) demonstrated that aerobic fluidized bed treatment was effective at low groundwater temperatures (4°C) for the biodegradation of chlorophenols. They reduced 2,4,6-TCP and 2,3,4,6-tetrachlorophenol (TeCP) by more than 99% and pentachlorophenol (PCP) by 83.5% over a period of 22 days. Hydraulic retention time was held constant at 5.0 hours. Chlorophenols were the only carbon source. The authors compared this method to biofilters with immobilized *Rhodococci* and physical adsorption onto activated carbon. They determined that the *Rhodococci* was not efficient at lower temperatures and that activated carbon reached its capacity in just a few weeks. An enrichment period of a few months was required to biodegrade PCP over 99% using an aerobic fluidized bed treatment. They determined that aerobic fluidized bed treatment was effective for on-site bioreclamation of chlorophenol-contaminated groundwater at high flow rates and lower temperatures.

Mäkinen et al. (1993) studied the bioremediation of simulated groundwater containing a chlorophenol mixture of TCP, TeCP, and PCP in a laboratory-scale aerobic fluidized-bed reactor. Chlorophenols were the sole source of carbon. They used a hydraulic retention time of 5.0 hours and a chlorophenol loading rate of 445 mg/L· d. They achieved a 99.7% reduction in chlorophenols and 94% of the theoretical mean

inorganic chloride release (ICl). The ICl increase and the decreases in pH from 7.2-7.3 to 6.9-7.0 indicated mineralization of TCP. Expected oxygen consumption further supported mineralization. They used the Microtox acute toxicity assay, where luminescent bacteria were exposed to the treatment effluent, to monitor the degradation of chlorophenols. The Microtox assay uses a suspension of the marine bacterium *Photobacterium phosphoreum.* The assay bacteria were fed known standard concentrations of chlorophenols to establish a dose-response curve. The Microtox test consisted of measuring the bioluminescence output of the assay bacteria and then comparing that to the bioluminescence output of bacteria not exposed to chlorophenols. They determined that toxicity increased with the degree of chlorination. To study the effect that an upset or disturbance had on the degradation performance, the aeration process was interrupted several times by withholding oxygen overnight. Two different chlorophenol feed mixtures were monitored: mixture #1, 25 mg/L of 2,4,6-TCP, 25 mg/L of 2,3,4,6-TeCP, and 40 mg/L of PCP; and, mixture #2, 35 mg/L of 2,4,6-TCP, 31 mg/L of 2,3,4,6-TeCP, and 27 mg/L of PCP. It took 30-40 days to overcome the upset each time the oxygen was withheld. They monitored the upset by changes in the PCP concentration in the effluent. The PCP concentrations were directly measured by gas chromatography. They concluded that the Microtox assay responded to changes in chlorophenol concentrations as low as 0.1 mg/L for PCP and that it could be used as an easy indicator for system upsets.

Järvinen et al. (1994) studied the effect that low-groundwater temperatures had on the bioremediation of chlorophenol. They inoculated laboratory-scale, continuous

flow reactors with nonacclimated activated sludge obtained from a chemi-thermomechanical pulp mill. They spiked the groundwater in the flow reactors with chlorophenol concentrations of 7-11 mg/L of 2,4,6-TCP, 32-36 mg/L of 2,3,4,6-TeCP, and 1.8-2.3 mg/L of PCP. The reactors were in a controlled-temperature incubator in the dark and the influent groundwater was at 4°C. A hydraulic retention time (HRT) of 5.0 hours resulted in effluent concentrations of less than 0.003 mg/L of each of the chlorophenol concentrations. Chlorophenol biodegradation was 99.9%. They concluded that TCP and TeCP were readily biodegradable, but that the chlorophenol degrading microorganisms needed to be acclimated and enriched for approximately 1.5 months to get efficient PCP degradation. This system can be operated and maintained at low groundwater temperatures which would eliminate the expense of having to heat the groundwater. Biodegradation of chlorophenols could take place in situ even at these low groundwater temperatures.

Immobilization of Cells for Biodegradation of Chlorophenols

The technique of using immobilized microbial cells as a biological wastewater treatment process has been used for many years. Examples of immobilized microbial systems for wastewater treatment include trickling filters, anaerobic fixed bed and fluidized bed systems. Entrapped microbial cells is another type of immobilization that has been studied because of its effective degradation of toxic organic compounds (Yang et al., 1989; Chibato and Tosa, 1977; Chibato and Tosa, 1983). The immobilization method entraps microbial cells in a three-dimensional porous gel lattice. The pores are

small enough to confine the cells and large enough for the diffusion of substrate and products to and from the cell (Mattiasson, 1983). Yang et al. (1989) investigated different carriers to entrap mixed microbial cells for removal of organics from wastewater. They operated their systems at different chemical oxygen demand (COD) loading rates and assessed the operational stability of the process. The polymeric materials tested included cellulose triacetate (mono-carrier), a combination of cellulose triacetate and calcium alginate (bi-carrier), polyacrylamide, and K-carrageenan. The two substrates used in the study were glucose and phenol. The bi-carrier was used to determine COD removal efficiency and effluent quality at various COD loading rates. The mono-carrier was used to determine long term operational performance because it had better mechanical strength. Concentrations of COD, suspended solids and NH_4-N were monitored. By using a scanning electron microscope, it was determined that the bi-carrier was more porous than the monocarrier. Also, it was observed that the bi-carrier was more elastic than the monocarrier. It was determined that calcium alginate was weak in mechanical strength, polyacrylamide was too complicated to prepare and it was also weak in mechanical strength. K-carrageenan was also weak in mechanical strength. Calcium alginate was determined to have better chemical oxygen demand (COD) removal efficiency and stable operational performance as compared to the other carriers tested. They did not investigate using polyvinyl alcohol (PVA) as a carrier.

Microorganisms that have been immobilized have been found to be better protected against toxicity than free cells. Bettman and Rehm (1984) entrapped the phenol-adapted *Pseudomonas sp.* in alginate and polyacrylamide-hydrazide (PAAH). An

airlift fermenter was used as a culture vessel. A sieve-like container within the fermenter held the immobilized cells. This process simulated entrapped microorganisms in a packed column. Continuous measurements of pH, optical density (OD), and O_2 concentrations were taken. They compared the phenol degradation activity and the cell growth of the entrapped microbial cells to those of free microbial cells. Results showed that free cells degraded phenol up to a concentration of 1.5 g/L and the entrapped cells in both alginate and PAAH degraded phenol up to a concentration of 3 g/L. They concluded that entrapment protects the microorganisms against the toxicity of phenol.

Keweloh et al. (1989) determined that the size of the microcolonies determines the extent of phenol tolerance. They supposed that the external cells would bind the phenol protecting the internal cells which continued to multiply without inhibition.

O'Reilly and Crawford (1989) immobilized *Flavobacterium* cells in polyurethane to biodegrade pentachlorophenol (PCP) in a batch study. They compared the PCP degradation activity of the immobilized cells to that of free cells at different concentrations. The experiments were performed in batch, semicontinuous batch, and continuous-culture bioreactors. Results showed that at initial concentrations below 100 mg/L PCP differences in degradation activity of free and immobilized cells were not detected. Immobilized cells were able to mineralize an influent concentration of 200 mg/L of PCP within 4 days, but the free cells were unable to mineralize PCP during the four-day course of the experiment. The semi-continuous batch experiment was fed a constant concentration of 10 mg/L PCP and ran for 150 days. An 86% removal of PCP was achieved during the first 15 day period, but the removal efficiency decreased to 12%

by the last 15 day period (days 136-150). The authors did not give an explanation for this decrease in removal efficiency. A 93% removal of PCP was achieved in the continuous-culture bioreactors within 30 days. The PCP influent concentration varied approximately from 5 mg/L to 15 mg/L. They concluded that twice the amount of PCP was degraded per gram of foam in the continuous-culture reactors than in the semicontinuous batch reactors. Polyurethane was determined to be an effective immobilization matrix as indicated by its protection against toxicity.

Sofer et al. (1990) studied the biodegradation of 2-chlorophenol (2-CP) using immobilized activated sludge. The activated sludge was a mixed microbial population that was acclimated to phenol over a period of 10 days. They were immobilized in sodium alginate and dropped into a calcium chloride solution to form 3 to 3.5 mm diameter beads. They used an air-sparged reactor to study system response and a recirculation reactor to study kinetic parameters. Physical removal of 2-CP, mainly by stripping, was monitored by control runs under identical conditions as immobilized cells, but without biomass. The rate constant for the physical removal by air stripping (K_3) was evaluated to be 0.085 hr^{-1} for the air sparged reactor and 0.057 hr^{-1} for the recirculation reactor. The temperature activity coefficient (O) was 1.16 indicating a high temperature dependence. The rate of biodegradation decreased as the spiking concentration of 2-CP increased. The maximum substrate utilization rate (K_m) decreased from 14.58 to 9.63 mg/L· hr corresponding to concentration increases from 50 to 100 mg/L. In the recirculation reactor, as the 2-CP concentration decreased, the pH decreased. As 2-CP concentrations decreased from 110 mg/L to 0 mg/L, pH values

correspondingly decreased from 5.5 to 4.2 pH. The authors did not explain the reason for the pH decrease. At a constant 2-CP concentration of 50 mg/L and at various biomass loadings from 30 g to 50 g, the corresponding K_m values were 3.8 to 8.3 mg/L. The half velocity coefficient (K_s) was 3 mg/L. They used the Monod expression for substrate utilization and modified it to include physical removal of substrate by stripping.

Valo et al. (1990) immobilized the chlorophenol-mineralizing *Rhodococci* bacteria on a polyurethane carrier. The polyurethane, seeded with *Rhodococci*, was placed in a glass column to form a biofilter. A concentration of 130 mg/L technical grade chlorophenol containing 2,3,4,6-TeCP, 2,4,6-TCP, and PCP was fed through the biofilter with added nutrients. The biofilter was aerated with pressurized air. The study compared the mineralization of PCP in a seeded biofilter and an unseeded biofilter. 40% of the PCP was recovered as CO_2 in the seeded biofilter and less than 1% of the PCP was recovered in the unseeded biofilter. They concluded that chlorophenols were degraded by immobilized bacteria without any additional carbon source and that the treated groundwater could be returned to the aquifer.

Hashimoto and Furukawa (1987) developed an inexpensive and effective new method for the immobilization of activated sludge. This new method is known as the polyvinyl alcohol (PVA)-boric acid method. The preparation of this method involved mixing one portion of concentrated activated sludge (mixed microbial cell population) with one portion of PVA aqueous solution. This mixture was dropped into a gently stirred saturated boric acid solution to form spherical beads. The beads were cured in the solution for 15-24 hours and then washed with tap water. The activated-sludge cells

were entrapped within a monodiol-type PVA-boric acid gel lattice. Monodiol-type PVA was shown as a single PVA compound with two hydroxyl groups. The beads produced were very durable with elastic, rubber-like properties. Continuous-treatment experiments were conducted using a synthetic wastewater to determine removal rates of total organic carbon (TOC) and total nitrogen (T-N). They also recorded the weight changes of the PVA-immobilized activated sludge beads. The TOC removal was 93% and T-N removal was 30-40%. The NO_3-N was denitrified in the anaerobic portion of the immobilized activated-sludge beads (aerated denitrification). Aerated denitrification was the contributing factor to the high removal rates of nitrogen. They thought the reason was because nitrifiers were also entrapped and could be maintained stably in the aeration tank due to being immobilized. The beads increased in weight as the loading increased, indicating growth of the microorganisms in the beads. In conclusion they determined that the PVA-boric acid method was inexpensive compared to other methods and that it was possible to operate an immobilized cell system at 2-3 times the loading rate of conventional systems. They did not address the problem of the tendency for the PVA beads to agglomerate. Also, they thought that the microbial activity was not reduced because of the low pH during the immobilization process where the beads are cured in the boric acid solution (pH 4) for 24 hours. Their reasoning was that activated sludge cells become surrounded by extracellular polymer, allowing them to withstand condition changes.

Wu and Wisecarver (1992) investigated and modified the PVA-boric acid method developed by Hashimoto and Furukawa (1987). They prepared the PVA beads using the

same method but added a small amount of sodium alginate to prevent or minimize the tendency for the beads to agglomerate. They entrapped a pure strain of phenol-degrading *Pseudomonas*. They demonstrated the viability of the immobilized cells by utilizing them in a fluidized bed bioreactor for a period of two weeks. Influent phenol concentrations from 250 to 1300 mg/L were continuously fed through the bioreactor achieving 100% removal. They determined that the removal of phenol was due almost entirely to biodegradation. Physical removal of phenol by stripping was measured by diverting the off gas through a NaOH solution and measuring the absorbed phenol. Physical removal was found to be less than 0.1% of the total phenol degraded. They tested the bead integrity in an 8-L fluidized bed column. The column was sparged with air at a rate of 1.4 L/min. The beads were able to withstand high shears with no sign of breakage. The authors suggested that this technique might be applicable to a wide variety of other microorganisms.

Stormo and Crawford (1994) developed a method to encapsulate bacteria and their nutrients in microbeads small enough to travel through aquifer material. They encapsulated *Flavobacterium* cells in agarose, forming microbeads with diameters of 2-50 μm. Aquifer material was packed into 24 columns. Some of the columns were sterilized by irradiation. In some of the columns free *Flavobacterium* cells were mixed with aquifer material, others had agarose microimmobilized *Flavobacterium* cells mixed with the aquifer material. Other columns had free *Flavobacterium* cells or agarose microimmobilized *Flavobacterium* cells injected into the aquifer material at 10 mL day^{-1}. The columns were all fed pentachlorophenol (PCP)-contaminated groundwater at various

in situ flow rates of 5.0 mL/day, 2.0 mL/day, and 12 mL/day. Effluent PCP levels were kept at near-detection limits by introduced bacteria until influent PCP concentrations exceeded 150 mg/L. The results showed that no noticeable difference occurred in the degradation rates between free and encapsulated cells in sterile or native aquifer material. The agarose carrier used to encapsulate the cells provided nutrients, a moisture reserve, and isolated them from predators. They concluded that immobilized cells' long-term survivability was enhanced over free cells.

Hanaki et al. (1994) investigated the applicability of using cells immobilized by the PVA-boric acid method in an anaerobic treatment process. They immobilized acetate-utilizing methanogens that are used in the anaerobic treatment process. These bacteria are sensitive to fluctuations in their environments, especially to influent quantity, wastewater constituents, toxic materials, and pH. Batch experiments were conducted under anaerobic conditions to determine the effect of toxic substances to the PVA immobilized methanogens. Various concentrations of toxic substances were added to vials containing the immobilized methanogens. The toxic substances used were phenol, sodium oleate (oleic acid), $NiCl_2$ (nickel), Na_2S (sulfide), propionic acid (propionate), and NH_4Cl (ammonia). They prepared the beads at different pHs by adjusting the pH with Na_2CO_3. The pHs ranged from 4.0 to 6.0. At a pH of 4, the initial lag phase prior to the active methane production was prolonged. The lag phase shortened as pH increased. The beads prepared at pH 4.0 began methane production after continuously feeding with acetate for 20 days. The beads made at pH 6.0 lost gel strength and had poorer durability. Therefore, they concluded that the production of beads at pH 4.0 was

the better immobilizing condition. The toxic effects of phenol, oleic acid and nickel were reduced and the authors concluded it was due to the adsorption of these substances by the bead material. The authors speculated that a pH gradient occurs within the beads, protecting the bacteria from acidic substances such as sulfide and propionic acid. The inhibitory effect of ammonia as compared to free cells was not reduced by either adsorption or formation of a pH gradient. It was observed that by increasing the ammonium concentration from 2000 to 6000 mg/L as nitrogen, retardation of methane production by unacclimated methanogens occurred in both the free and immobilized systems. The immobilized system had no advantage over the free cell system for the prevention of ammonia toxicity because a higher pH within the bead does not relieve the inhibitory effect by free ammonia.

Permeable Barriers and Different Mediums Used

Thomson et al. (1991) examined the concept of designing permeable barriers to stabilize, remove, or degrade groundwater contaminants in situ. Permeable barriers were applicable to either shallow aquifer systems (<20 m) which are accessible by trenching equipment, or deep aquifer systems which are accessible by wells. Permeable barriers constructed by trenching had two advantages: 1) accessibility of the medium placement and 2) ease of recovery of medium by re-excavation. Permeable barriers were further classified as either passive or active. An active barrier required continuous operation and maintenance. A passive barrier required little or no operation or maintenance once the medium is in place. Two trench-based permeable barriers were analyzed. An in situ air

stripper was an active barrier and consisted of a perforated pipe placed in the bottom of the trench and then the trench was filled with crushed limestone. Air was bubbled through the groundwater and limestone, stripping volatile compounds. The authors compared in situ air stripping with conventional packed tower air stripping and determined that 1) the trench-based stripping need high pressure air compressors, but no water pumping equipment was needed, making operating costs less and 2) biostimulation did occur from the oxygen, resulting in a combined air stripping and biodegradation of volatile organic contaminants. A geochemical barrier for immobilizing metals from uranium milling tailings was described as an example of a passive permeable barrier. This permeable barrier concept consisted of using limestone and peat as the barrier medium that neutralized the acidic leachate solution. The soluble inorganic contaminants were immobilized on the medium. Upon exhaustion it was re-excavated and disposed of as a hazardous waste. The authors concluded that the permeable barrier concept had several advantages which include reduced capital, and operations and maintenance costs, improved reliability, and less volume of treatment by-products. Another advantage the barrier treatment process had over conventional surface processes was that it operated at much lower process loading rates due to low groundwater velocities and low contaminant concentrations in groundwater. Therefore, the authors determined that low cost natural medium materials such as gravel, limestone, and peat could be utilized to achieve treatment. This paper focused more on physical and chemical means rather than biological.

Morrison and Spangler (1993) further explored the concept of using chemical

barriers as a low-cost means to protect groundwater. They described a chemical barrier as a passive in situ water-treatment system. Chemicals used in the barrier were placed in the subsurface either by lining a disposal site, by trench and fill, or by injection. Dissolved contaminants became part of the immobile solids of the aquifer, by either precipitation or adsorption, as the contaminated groundwater passed through the chemical barrier. They conducted column studies on a precipitation barrier and a sorption barrier to remove metals from uranium milling tailings. They determined that a high pH was needed to precipitate heavy metals as hydroxides. They concluded that accurate groundwater characterization was more critical in determining the performance of sorption barriers than precipitation barriers, because each contaminant has its own pH for optimal adsorption. Removal was the result of a chemical reaction, not a biological reaction.

Morrison (1995) conducted laboratory batch and column studies on chemical reactive barriers for the purpose of evaluating the applicability for in situ remediation of uranium tailings. He examined sorption capacities of various contaminants under aquifer flow conditions on barriers containing low cost materials. Removal rates for uranium and molybdenum were greater than 99% and 96%, respectively. It was determined that ferric oxyhydroxide can immobilize metals and uranium and that it is (1) inexpensive, (2) injectable, (3) remained immobile after emplacement, and (4) did not reduce the aquifer's permeability. This was described as an abiotic system and removal was attributed to sorption. There was no investigation as to the possibility of biological removal.

Rumer and Ryan (1995) determined that trench excavation was usually to a depth of 20-30 meters using a backhoe. The backhoe created a trench 0.5-2.0 meters in width. The backhoe was the least costly method and the most rapid. A dragline or clamshell attached to a crane was used for trenches to depths of 50 to 75 meters, respectively. The clamshell created a trench 0.3-2.0 meters in width and the dragline created a trench 1.0-3.0 meters in width. The clamshell and dragline were slow and used for wide, deep excavations.

Rael et al. (1995) investigated the feasibility of an in situ permeable barrier to remove benzene from groundwater by adsorption onto a variety of medium materials. This technology was limited to the depth accessible by trenching equipment and therefore was applicable to shallow aquifer systems of less than 30 meters. The author suggested that, if necessary, slurry walls or sheet piles could be strategically placed to funnel the contaminant plume through the permeable barrier. It was concluded that a mix of powdered activated carbon (PAC) and sand would be a successful media. The barrier medium allowed the flow of water but adsorbed with the contaminant preventing further migration. The authors stated when the barrier reached its treatment capacity it can be replaced with fresh media.

O'Hannesin (1995) field tested an in situ semipassive permeable reaction wall in which nutrients were introduced to enhance biodegradation of organics. The wall was installed across the path of a plume contaminated with trichloroethylene (TCE) and carbon tetrachloride. A stable anaerobic microbial population was produced some distance downgradient from the wall. The carbon tetrachloride was removed by the

anaerobic microorganisms but the TCE was not removed. This is an ongoing study and it is being further researched. No study was done on an aerobic system.

Diffusion Models for Polymer Matrixes

Tanaka et al. (1984) investigated the diffusion characteristics of several substrates with different molecular sizes into and out of calcium alginate beads. They used Crank's (1975) equation for diffusion into a sphere. The diffusion coefficients were obtained from the change in concentration of the substrates in a well-stirred solution. Diffusion values for substrates with molecular weights less than 2×10^4 agreed with those in the water system and could diffuse freely into and out of the gel beads. But no diffusion into the bead was observed from substrates with higher molecular weights such as albumin (MW = 6.9×10^4), γ-globulin (MW = 1.54×10^5) and fibringer (MW = 3.41×10^5).

Chen et al. (1993) used a linear adsorption model (LAM) to calculate diffusivities of Cu^{2+} in calcium alginate gel beads. They ran batch studies on calcium alginate beads prepared from 2%, 3%, 4%, and 5% sodium alginate solutions. Each different density bead was exposed to solutions containing concentrations of 0.01 M KCl, 0.001 M Ca^{2+}, and 0.001 M Cu^{2+}. The LAM model assumed that the reaction or exchange rate was faster than the rate of diffusion and that a dynamic equilibrium was established at every point throughout the bead. They compared the results from the LAM to the shrinking core model (SCM). The SCM was based on the observation that when examining a cross section of a partly reacted solid particle that the unreacted core of material was surrounded by an outer layer of reacted material. The metal ions diffused through the

transformed shell material to an unreacted core that was progressively shrinking. The authors concluded that the SCM and LAM took diffusion and chemical reaction into account, but found some critical differences. The assumptions in the SCM were: 1) chemical reaction rate exceeded the metal ion diffusion rate; 2) the concentration of diffusing metal ions approached zero at the surface of the shrinking core where the reaction took place; 3) the outer reacted shell is inert to the diffusing metal ions; 4) the shrinkage of the unreacted core is slower than the mass transfer of metal ions toward the core. The LAM assumed that: 1) the chemical reaction rate exceeds the metal ion diffusion rate; 2) absorption is linear; and, 3) some unoccupied reaction sites are available for immobilization. The LAM was applied to spherical beads and the authors advised that it was not limited to this situation and could be applied to metal ions in ion exchange resins.

Jang (1994) reevaluated the SCM calculating the diffusivity of Cu^{2+} in calcium alginate beads and compared those results to the LAM results. He determined that Chen et al. (1993) had not evaluated the SCM slope correctly. Rao and Gupta (1982) reported that in using the SCM that data points at large times deviate from the initial straight line. They thought that the deviation was caused by (1) the increasing sensitivity of $F(X) = 1 - 3(1 - X)^{2/3} + 2(1 - X)$ as the value of X approached 1 and that any introduced experimental error contributed to the uncertainty of F(X); and, (2) as the reaction front moved toward the unreacted core of the sphere, the reacted outer shell thickens and became increasingly difficult for a new concentration profile to develop without a time delay. The value of X was described as the extent of the reaction, $[C_0 - C]/[C_0 - C_\infty]$,

where C was the concentration of free metal in solution, C_0 was the initial concentration of free metal in solution, and C_∞ was the concentration of free metal in solution at equilibrium. Jang (1994) concluded that the SCM was easier to apply than the LAM and that the SCM gave good approximate diffusivity values.

CHAPTER III

MATERIALS AND METHODS

Experimental Approach

This study focused on evaluating PVA-immobilized cells as a permeable barrier medium for in situ bioremediation of TCP contaminated groundwater. Beads were prepared in accordance with the PVA-boric acid method (Hashimoto and Furukawa, 1987; Wu and Wisecarver, 1992) using various molecular weights (MW) of PVA to obtain a porous, rubber-like, elastic bead for the purpose of immobilizing cells and using it as a permeable barrier medium. A bed of beads was characterized with its density, porosity, permeability, and compressibility or deformation. Batch studies were conducted to obtain necessary data to determine the rate of TCP diffusion into the PVA beads, adsorption properties of the beads, and the substrate-use rate of the mixed bioculture as free cells and as immobilized cells. An initial column study was conducted with a constant concentration of TCP fed at a constant flowrate into a column with a packed bed of PVA-immobilized cells. The bed of PVA beads was located between layers of aquifer sand. The purpose of this experiment was to simulate a "bio-trench" and monitor the biodegradation rate of TCP. A final column study was conducted with a constant concentration of TCP fed at a constant flowrate into two columns. The two

columns varied in size to provide different hydraulic retention times (HRT) to show the effect on biodegradation rates.

Chemicals

Polyvinyl alcohol (MW 88,000, 98% hydrolyzed; MW 115,000, 97.7% hydrolyzed; MW 126,000, 98% hydrolyzed) was obtained from Scientific Polymer Products, Inc., Ontario, NY. Alginic acid, sodium salt (low viscosity) was obtained from the Sigma Chemical Co., St. Louis, MO. 2,4,6 trichlorophenol (TCP) was obtained from Fluka Chemical Corp., Ronkonkoma, NY. 2,4,6 tribromophenol (TBP) was obtained from Acros Organics, New Jersey. Ethyl acetate and methanol were obtained from Fisher Scientific. Potassium bromide was obtained from J. T Baker Chemical Co., Phillipsburg, NJ. All chemicals used in this study were reagent grade.

A dilute solution of TCP was prepared by dissolving 10.0 g of 2,4,6-TCP in 0.02 N NaOH to make a 1.0 liter solution with a final concentration of 10.0 g/L. The 0.02 N NaOH solution was prepared with distilled water (Standard Methods, 1975). A dilute solution of TBP was prepared by dissolving 5.0 g of 2,4,6-TBP in a 0.02 N NaOH to make a 1.0 liter solution with a final concentration of 5.0 g/L.

Groundwater Analysis

Groundwater was obtained from a water well located in the NE/4 NE/4 NE/4 Section of 9-T16N-R2E, Lincoln County, Oklahoma. The groundwater was initially

analyzed by the State of Oklahoma, Department of Environmental Quality, Water Laboratory, and the Total Organic Carbon (TOC) was analyzed by The Stover Group, Analytical/Toxicology Laboratories, Stillwater, Oklahoma. Standard EPA analytical methods were used in accordance with federal regulations (40 CFR 136). The groundwater analysis is given in Table 8, Appendix B.

Preparation of PVA Beads

The polyvinyl alcohol (PVA)-boric acid method developed by Hashimoto and Furukawa (1987) and modified by Wu and Wisecarver (1992) was used to prepare "blank" PVA beads. The "blank" beads were prepared without microorganisms for the purpose of comparing beads made with different molecular weights of PVA. Distilled water was added to 43.7 g of PVA (MW 88,000; MW 115,000; and MW 126,000) to obtain a 330 mL solution. A 13% w/v (43.7 g/330 mL) ratio was maintained as recommended by Hashimoto and Furukawa (1987) and by Wu and Wisecarver (1992) for best bead strength and formation. The solution was heated to 60°C and stirred constantly until the PVA was dissolved. A 3.5 mL volume of a 2% sodium alginate solution was added to the 330 mL PVA solution. The 2% w/v sodium alginate solution was made by adding distilled water to 0.5 g of alginate acid (sodium salt) to obtain a 25 mL solution. The 2% sodium alginate solution was continuously stirred for about 30 minutes until dissolved. The PVA-sodium alginate solution was cooled to 35°C. A 30 mL volume of distilled water was added to the solution and stirred thoroughly. The solution was then drawn through tygon tubing (ID 3.1 mm) by a peristaltic pump (Cole-Parmer 7553-

30) and extruded through a tubing connector with a 1.0 mm diameter opening inserted into the end of the tubing. As droplets formed, they fell into a gently stirred boric acid solution to form beads. The boric acid solution was made by adding hot water (40°C) to 250 g H_3BO_3 and 20 g CaCl to obtain a 1.0 liter saturated solution. The saturated boric acid solution was then cooled to room temperature. Some boric acid did precipitate out. This ensured a saturated solution. The beads were cured in the gently stirred boric acid solution for 24 hours. The beads were then rinsed thoroughly in distilled water several times to remove all of the boric acid solution. The beads were left soaking for about an hour, rinsed again in distilled water, and then drained.

Microorganisms

Activated sludge obtained from the Georgia-Pacific Leaf River Pulp Mill, New Augusta, Mississippi, was used in this study. The mill operation included a bleaching process. According to WHO (1989) chlorophenols can unintentionally be produced from a chlorine bleaching process in pulp and paper-mills. Microorganisms from this type of mill were assumed to have had some exposure to chlorophenols and therefore capable of being quickly acclimated for the purpose of this project. The activated sludge was obtained from the recirculation line where there was a high cell concentration. The activated sludge was shipped and received within 24 hours. The microorganisms were acclimated by feeding them 10.0 mg/L TCP as their sole carbon source over a period of 10 days with continuous aeration and addition of nutrients. 1 mL of each of the following nutrient solutions was added to each liter of activated sludge (Standard

Methods, 1975; Method 507):

- *Phosphate buffer solution.* 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl were dissolved in distilled water and then diluted to 1 liter.
- *Magnesium sulfate solution.* 22.5 g $MgSO_4 \cdot 7H_2O$ were dissolved in distilled water and then diluted to 1 liter.
- *Calcium chloride solution.* 27.5 g $CaCl_2$ were dissolved in distilled water and then diluted to 1 liter.
- *Ferric chloride solution.* 0.25 g $FeCl_3 \cdot 6H_2O$ were dissolved in distilled water and then diluted to 1 liter.

A standard ratio of the weights of carbon (C), nitrogen (N), and phosphorus (P) was used to ensure that microorganisms were receiving minimal amounts of nutrients and carbon for growth. The standard ratio used for C:N:P was 100:10:3 (Beltrame et al., 1984). The amounts of carbon, nitrogen and phosphorus provided to the microorganisms in this study are shown in Table 1 and the weight ratio of C:N:P was 100:18:188.

TABLE 1.

NUTRIENT REQUIREMENTS

Nutrients	Amount from TCP (mg/L)	Amount from Groundwater (mg/L)	Amount from Nutrient Solution (mg/L)	Total Amount (mg/L)	Ratio
Carbon Source (C)	3.65	1.5	0	5.15	100
Nitrogen Source (N)	0	0.5	0.4449	0.9449	18
Phosphorus Source (P)	0	0	9.7	9.7	188

Cell Immobilization

The activated sludge was centrifuged using an International Equipment Co. Clinical Centrifuge for 10 minutes at 4000 rpm to obtain biomass for immobilization. To determine the amount of volatile suspended solids (VSS) from the centrifuged sludge, 5.2805 g centrifuged wet weight of biomass was ignited at 550°C in a muffle furnace for 30 minutes, cooled to room temperature in a desiccator and then weighed (Standard Methods, 1975; Method 208E).

The polyvinyl alcohol (PVA)-boric acid method developed by Hashimoto and Furukawa (1987) and modified by Wu and Wisecarver (1992) was used to immobilize 43.7 g centrifuged biomass. The procedure is outlined in Figure 2. Distilled water was added to 43.7 g of PVA (MW 88,000) to obtain a 330 mL solution. The solution was heated to 60°C while stirring constantly until the PVA was dissolved. A 3.5 mL volume of a 2% w/v sodium alginate solution was added to the PVA solution. The PVA-sodium alginate solution was cooled to 35°C. The centrifuged cells (43.7 g wet weight) and 10 mLs distilled water mixed with 1.3 mLs of nutrient medium were added to the cooled PVA-sodium alginate solution and stirred thoroughly. The solution was then drawn through tygon tubing (ID 3.1 mm) by a peristaltic pump (Cole-Parmer 7553-30) and extruded through a tubing connector with a 1.0 mm diameter opening inserted into the end of the tubing. As droplets formed, they fell into a gently stirred boric acid solution to form beads. The beads were cured in the gently stirred boric acid solution for 24 hours. The beads were then rinsed and soaked thoroughly in distilled water several times to remove all of the boric acid solution.

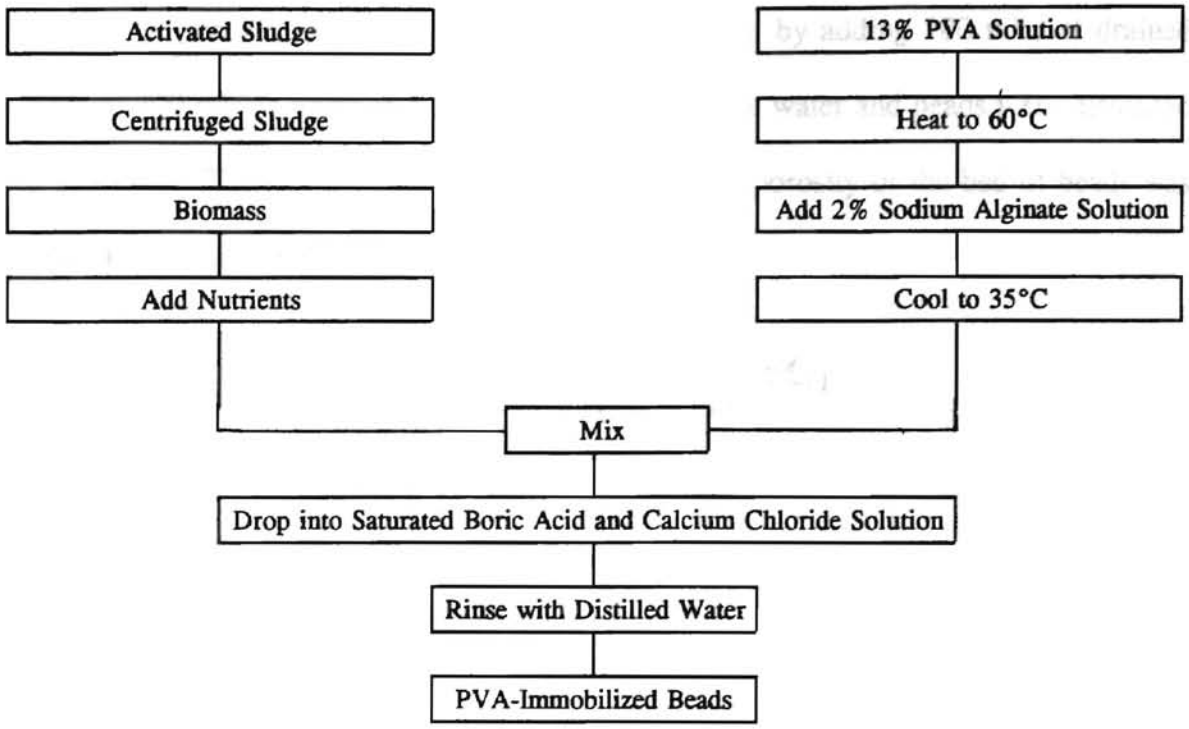


Figure 2. PVA-boric acid method to immobilize cells.

Characterization of a Packed Bed

Specific Gravity Determination

The density (mass/unit volume) of a bed of beads and water were determined at 22°C by weighing 100 mLs of drained beads and 100 mLs of water. The expression to determine the specific gravity of the bed of beads was given as (Smith, 1979):

$$Specific\ gravity = \frac{Density\ of\ Substance}{Density\ of\ Water} \qquad (1)$$

Porosity

The porosity of a bed of beads was determined by adding 100 mLs of drained beads to 100 mls of water in a graduated cylinder. The water and beads were displaced to 175 mLs. The expression to determine the percent porosity of the bed of beads was given as (Smith, 1979):

$$\% \ Porosity = \left[\frac{(V_1+V_2)-V_3}{V_1}\right](100) = \frac{Total\ Void\ Volume}{Total\ Volume}(100) \qquad (2)$$

Where
V_1 = volume of beads
V_2 = volume of water
V_3 = volume of displacement

The aquifer sand was obtained from the Oklahoma State University Agronomy Research Station and its particle size was determined to be between 20-40 U.S. Standard sieve size. The porosity of a bed of sand was determined by packing 160 g of sand into a 100 mL volume in a graduated cylinder. A volume of 100 mLs of water was added to the graduated cylinder. Equation (2) was used to determine the porosity of the sand. The water and sand were displaced to 170 mLs.

Compressibility Study

An oedometer (or consolidation test apparatus) was used to determine the compression behavior of a packed bed of PVA beads. The oedometer cell was 70 mm in diameter and 9 mm in height (Figure 3). The bottom of the cell was lined with a

sheet of clear plastic wrap. The cell was packed with PVA beads (Figures 4 and 5) and distilled water was added to the cell to simulate beads in a saturated zone. A sheet of clear plastic wrap was placed on top of the PVA beads in an attempt to contain the water within the cell. The cell was placed under the initial vertical load of the oedometer (Figure 6). The applied force was gradually increased to 1.28 tons/ft^2 over a period of 500 minutes to simulate the overburden pressure equivalent to that found at the bottom of a 40.0 ft trench filled with PVA beads. Consolidation of the bed was quantified in inches and read from the oedometer gauge in 1.0 minute increments. Figure 7 shows the compressed bed of beads after the load was removed. The compression index was calculated from the expression (Smith, 1979):

$$C_c = \frac{\Delta V}{V} \tag{3}$$

Where
Cc = Compression Index
ΔV = Change in Volume of Bed
V = Initial Volume of Bed

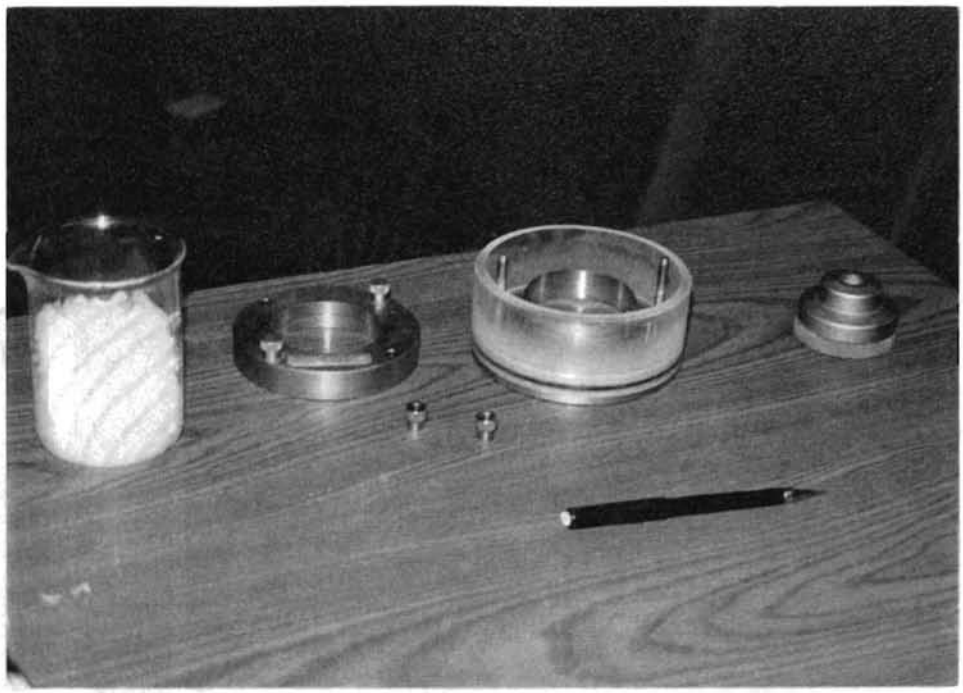


Figure 3. Oedometer cell.

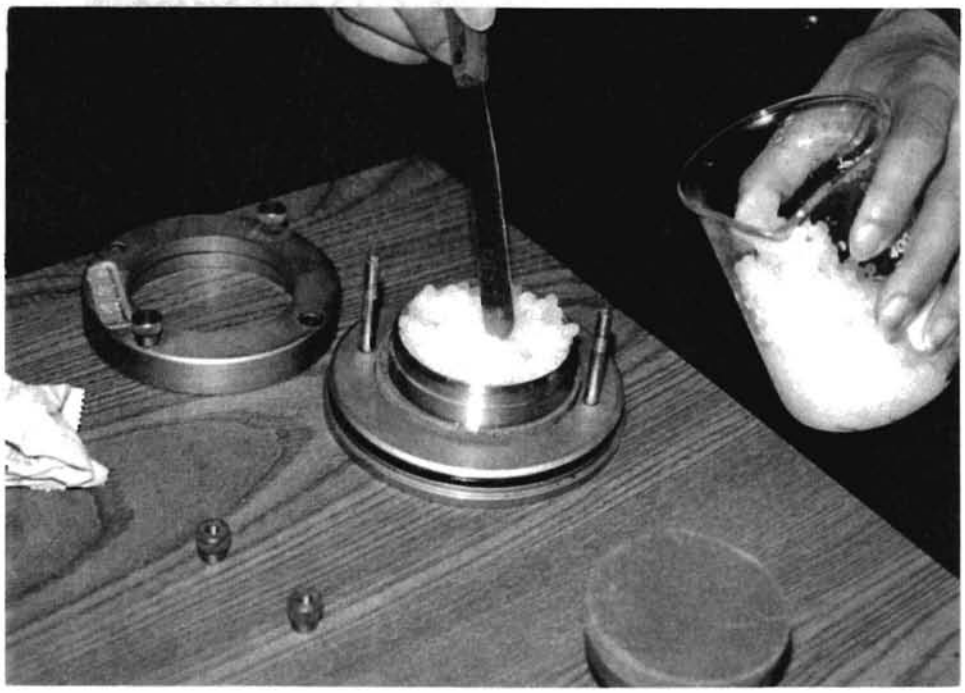


Figure 4. Packing PVA beads into oedometer cell.

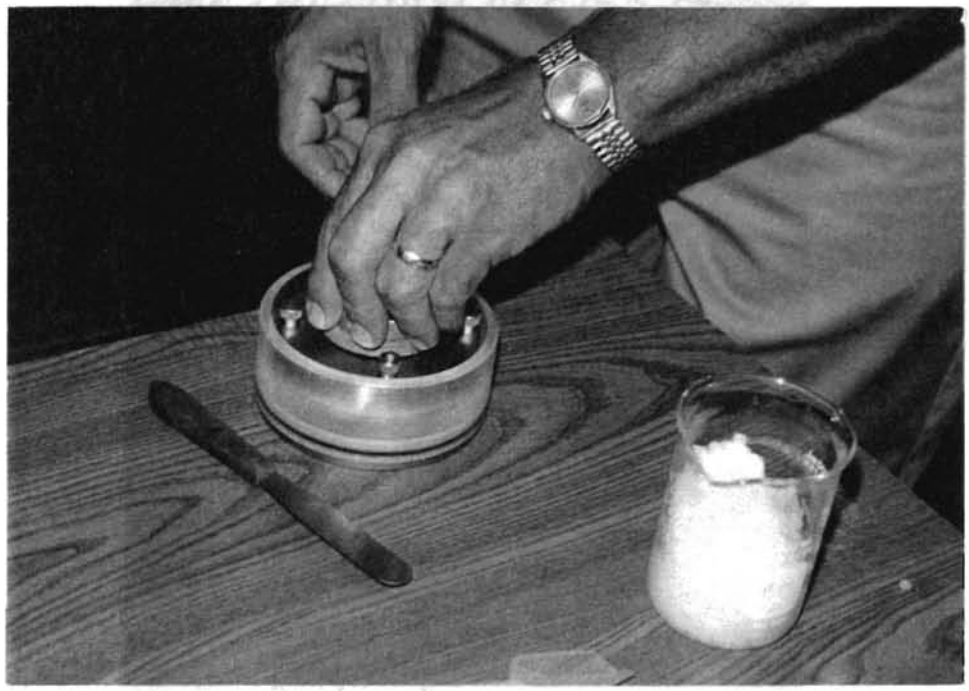


Figure 5. Placing packed oedometer cell into ring.

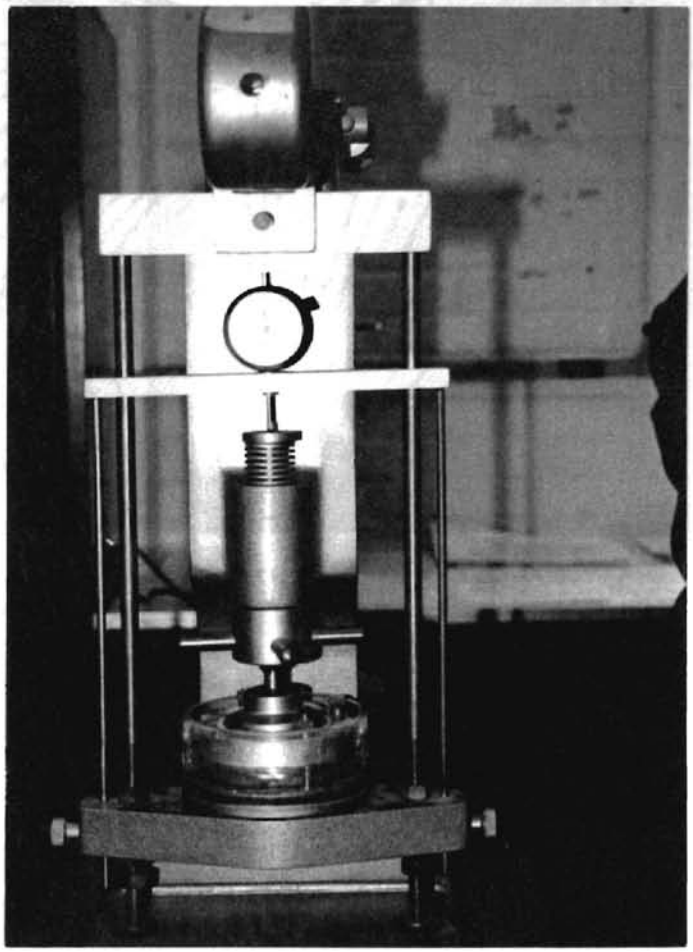


Figure 6. Vertical load was placed on oedometer cell.

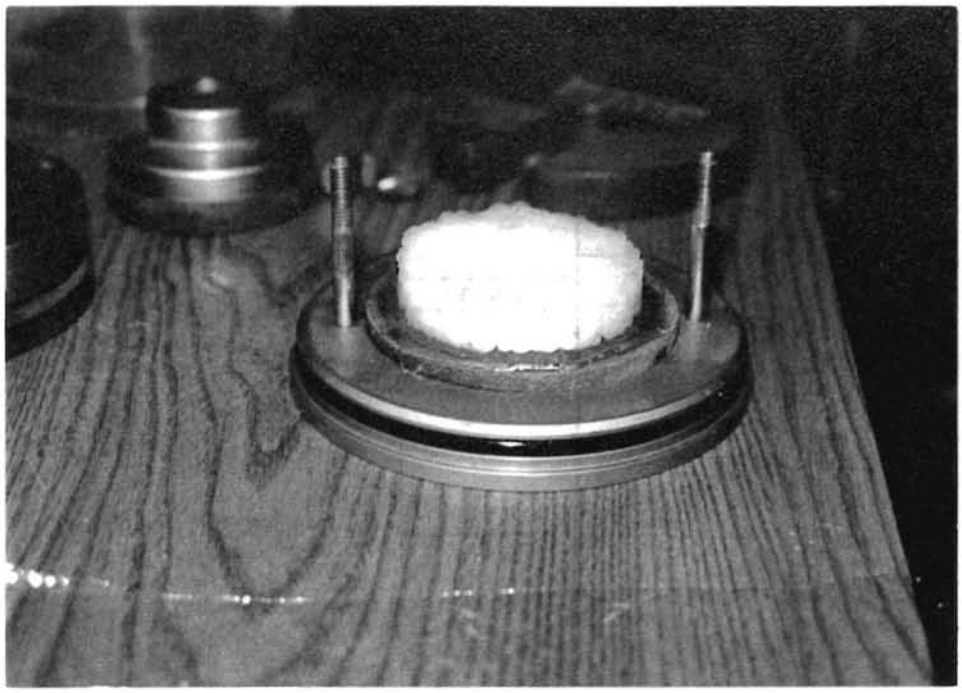


Figure 7. Compressed bed of PVA beads after load removed.

Permeability Study

The falling head permeameter test was used to determine the permeability coefficient (K) of a packed bed of PVA beads (Smith, 1979). An illustration of the falling head permeameter is shown in Figure 8. PVA beads were drained and packed into the bottom portion of a column for the length of sample (L). Two (2) copper screens were placed above and below the layer of beads. A shallow layer of washed gravel was placed on top of the beads to hold them in place. A graduated cylinder was placed below the column to catch the flow of water. Water was added to the column to a certain height (h_1). The valve was opened at the bottom and the stop clock was started. After a measured time (t), the height to which the water had fallen (h_2) was determined.

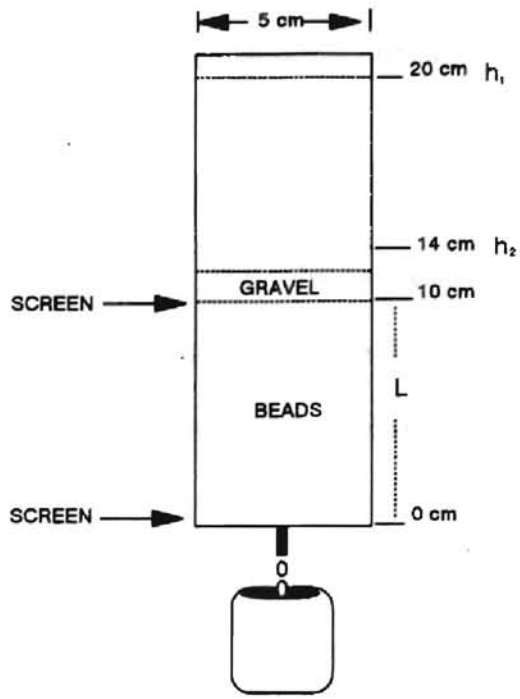


Figure 8. Falling head permeameter test.

The permeability coefficient was determined by the expression (Smith, 1979):

$$K=2.3\ \frac{L}{t}Log_{10}\ \frac{h_1}{h_2} \qquad (4)$$

Where
K = permeability coefficient
L = length of sample
t = time
h_1 = height of water
h_2 = height of which water level has fallen

The falling head permeameter test was used to determine the permeability coefficient (K) of a packed bed of aquifer sand (Smith, 1979). Sand was packed into the

bottom portion of a column at a density of 1.6 g/cm^3 for the length of sample (L). Two (2) copper screens were placed above and below the layer of sand. A shallow layer of washed gravel was placed on top of the sand to hold the grains in place. A graduated cylinder was placed below the column to catch the flow of water. Water was added to the column to a certain height (h_1). The valve was opened at the bottom and the stop clock was started. After a measured time (t), the height to which the water had fallen (h_2) was determined. Equation (4) was used to determined the permeability coefficient for the sand.

Batch Studies

Batch studies were conducted to obtain necessary data to determine the rate of TCP diffusion into PVA beads, adsorption properties of the beads, and the kinetic substrate use rate of the mixed bioculture as free cells and as immobilized cells.

Adsorption Studies

Blank beads made with PVA (MW 88,000) were prepared as previously described and cured for 24 hours. The beads were thoroughly rinsed in distilled water. The beads were soaked in distilled water and then rinsed again. An initial equilibrium study was conducted on 50.0 g of blank PVA beads in a continuously stirred 500 mL volume of 20.0 mg/L TCP solution. Samples of the solution were taken over a 24 hour period and analyzed for TCP concentrations to establish an equilibrium time. The adsorption study consisted of adding varying masses of drained beads (0.0 g, 1.0 g, 5.0 g, 10.0 g, 15.0 g, and 20.0 g) to 5 glass flasks of 250 mL volumes. A 100 mL volume of a 24.0 mg/L

TCP solution was poured into each flask. Flasks were covered to prevent photolytic degradation and were shaken for 24 hours. 3.0 mL samples were taken from each flask at 0.0 hours and at 24.0 hours to measure TCP concentrations. Data were analyzed by plotting as an isotherm.

An adsorption study was conducted on the 200-sieve mesh copper screen used in both the initial and final column studies. A 5.0 cm diameter circle of copper screen was placed in 100 mLs of 10.0 mg/L TCP solution and was shaken for 24 hours. 1.0 mL samples were taken at 0.0 hours, 3.0 hours, 7.0 hours, 12.0 hours, and 24.0 hours to measure TCP concentrations.

An adsorption study was conducted on the aquifer sand used in the initial column study. A flask containing 100 g sand and 100 mLs of 10.0 mg/L TCP solution was shaken for 24 hours. 2.0 mL samples were taken at 0.0 hours, 3.0 hours, 7.0 hours, 12.0 hours, and 24.0 hours to measure TCP concentrations.

Diffusivity Study

Blank beads were prepared as previously described and cured for 24 hours. The beads were thoroughly rinsed in distilled water. The beads were soaked in distilled water and then rinsed again. A reactor was set up as shown in Figure 9 by placing a 1.0 liter glass beaker into a water bath. 499.5 mLs of groundwater was poured into the reactor. A 50.0 g weight of blank beads (732 beads) occupied a volume of 70 mLs. The average diameter of 10 PVA beads was 3.8 $\pm$ 0.36 mm (SD). The 50.0 g of blank beads were added to the groundwater in the reactor. The beads and water were gently stirred continuously throughout the experiment. The solution was brought to equilibrium by

maintaining a constant temperature of 28°C, conductivity of 1040 μmhos/cm, and a pH of 7.8 for a period of 6.0 hours. A conductivity meter and probe were used to measure the conductivity. Conductivity of a solution is a measurement of its ability to conduct a current which is attributable to the ions in solution (Sawyer et al., 1994). The constant conductivity measurement ensured that the groundwater and beads had reached equilibrium including any osmotic pressure that could effect the rate of diffusion. A 0.5 mL volume of the 10 g/L TCP solution was added to the reactor and mixed thoroughly and quickly to ensure an initial TCP concentration of 10 mg/L. Samples of the solution were taken at various times over a 300 minute period to measure TCP concentrations. The reactor and collected samples were kept covered to prevent degradation by photolysis.

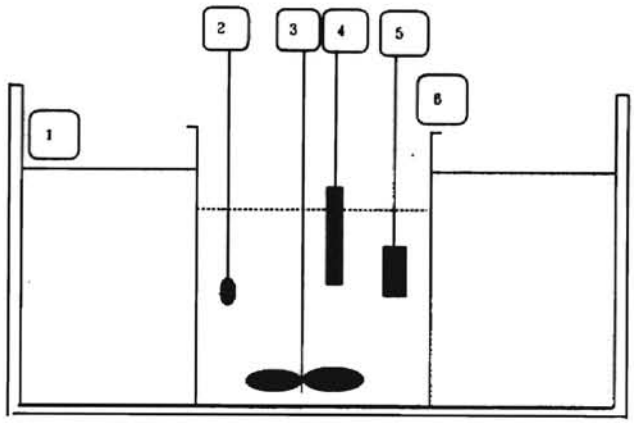


Figure 9. Schematic diagram of reactor system. (1) water bath; (2) thermometer; (3) motorized stirrer; (4) pH electrode; (5) conductivity meter; and, (6) glass vessel.

Kinetic Studies

Immobilized Cells

A batch culture of activated sludge had been acclimated for 60 days by feeding it 10 mg/L of TCP and nutrients each day. The culture was continuously aerated. The activated sludge was then centrifuged to concentrate the biomass. An International Equipment Co. Clinical Centrifuge was used to centrifuge the sludge at 4000 rpm for 10.0 minutes. The wet centrifuged biomass weighed 7.2 grams. The VSS of the centrifuged biomass was approximately 259 mg. This approximation was made from the previous analysis where 0.1905 g VSS was determined from 5.2805 g of centrifuged biomass (0.036 g VSS/g centrifuged biomass). The biomass was entrapped in 1/6th of the PVA recipe used to immobilize cells as previously described, keeping a 1:1 ratio of cells to PVA. The immobilized cells were cured for 24 hours. The immobilized cells were not fed any TCP during this period. The beads were rinsed in distilled water.

A batch study was set up by placing the immobilized cells in a flask. A 1.0 L feed solution made with groundwater spiked with 10.0 mg/L TCP and added nutrients was aerated for 15 minutes. The aerated feed solution was added to the flask. The flask was covered to prevent photolytic degradation and gently shaken. Samples of the solution were taken at various times to measure TCP concentrations. The experiment was conducted until 100% removal of TCP was reached, which took a period of 72.0 hrs. At the end of 72.0 hours the PVA-immobilized cells were drained and rinsed with groundwater. The experiment was conducted a second time by adding a fresh 1.0 L volume of 10.0 mg/L TCP aerated feed solution to the rinsed beads. Samples of the

solution were taken at various times to measure TCP concentrations. A 100% removal of TCP was reached within 24 hours. At the end of 24.0 hours the PVA-immobilized cells were drained and rinsed. The experiment was conducted a third time by adding a fresh 1.0 L volume of 10.0 mg/L TCP aerated feed solution to the rinsed PVA-immobilized cells. Samples of the solution for the third experiment were taken at 0.0 hrs, 1.0 hrs, 3.0 hrs, 5.0 hrs, and 8.0 hrs to measure TCP concentrations. The same experiment was conducted three times in a series because it took the bacteria several days to overcome the effects of the immobilization process.

The PVA-immobilized cells were drained and placed back into their original flask and a fresh aerated feed solution was added to the beads in the flask. A 50.0 mL sample volume was taken at 0.0 hrs, 1.0 hrs, 3.0 hrs, 5.0 hrs, and 8.0 hrs to measure the inorganic chloride ions (ICl). The inorganic chloride ion concentrations were determined by titration using the 408B Mercuric Nitrate Method from *Standard Methods* (1975).

The PVA-immobilized cells were drained and rinsed with groundwater. The PVA-immobilized cells were placed into a 300 mL BOD bottle to measure oxygen consumption. A 300 mL volume of 10.0 mg/L TCP aerated feed solution was added to the beads in the BOD bottle. A glass stopper and plastic cap were placed on the BOD bottle. The dissolved oxygen (DO) was measured at 0.0 hrs, 1.0 hrs, 3.0 hrs, 5.0 hrs, 8.0 hrs, and 24.0 hrs using an ORION Research Analog pH Meter (Model 301) and an O_2 electrode (ORION Model 97-08-00).

The PVA-immobilized cells were drained and placed back into their original flask.

A fresh aerated feed solution with TCP was added to the flask. The feed solution was changed daily for the remainder of the 45 day experiment. The batch study was terminated after 45 days to examine the beads with a scanning electron microscope (SEM).

Free Cells

A batch culture of activated sludge had been acclimated for 60 days by feeding it 10 mg/L of TCP and nutrients each day. The culture was continuously aerated. Prior to beginning the kinetic study on the free cells, an initial volatile suspended solids (VSS) analysis was conducted according to Method 208E, *Standard Methods* (1975). As a result of a VSS analysis of 4376 mg/L VSS, it was determined that the activated sludge needed to be diluted for the kinetic study. A 1.0 L volume of the activated sludge was poured into an 8.0 L bottle and diluted by adding 2.0 L of tap water. The 3.0 L batch culture was aerated continuously. The 3.0 L batch of free cells were fed 10.0 mg/L TCP and nutrients. A 100.0 mL sample volume was taken at 0.0 hour, 3.0 hours, 6.0 hours, 9.0 hours, 14.0, hours, and 22.0 hours. Each 100 mL sample was filtered under vacuum using a Whatman glass fiber filter. A 25.0 mL volume of the filtered sample was used for solid phase extraction for GC analysis. A 50.0 mL volume of the filtered sample was used for ICl analysis. The filtered residue was used for VSS analysis. Each of these analytical methods are further described later in this chapter under "Analytical Methods."

Column Studies

Initial Column Study

This experiment was carried out in a cylindrical acrylic column, and was set up as an aerobic, continuous flow packed-bed reactor (Figure 10). The column had an inside diameter of 5.0 cm and a height of 20.0 cm. The column had 5.0 cm beds of aquifer sand above and below a 10.0 cm packed bed of drained PVA-immobilized cells. The sand was packed at a density of 1.6 g/cm^3 to simulate a density similar to aquifer densities. The PVA-immobilized cells were packed at a density of approximately 0.9869 g/cm^3. A 5.0 cm diameter 200-sieve mesh copper screen was placed between the beds of sand and beads. A peristaltic pump (Cole-Parmer 7553-30) with a head (Model 7013) and tygon tubing (ID 0.8 mm, No. 6409-13) was used to pump the groundwater into the

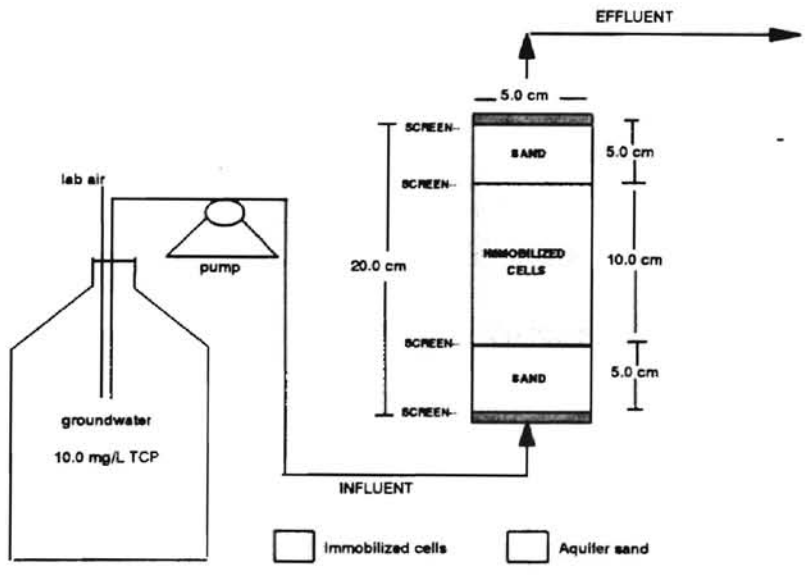


Figure 10. Schematic diagram of initial column study.

columns. TCP spiked groundwater was prepared in 25.0 liter glass bottles and covered with aluminum foil to prevent photolytic degradation. Groundwater was spiked with TCP to a final concentration of 10.0 mg/L. Nutrients were added in the amount of 1.0 mL per liter of each of the following solutions previously described: phosphate buffer solution; magnesium sulfate solution; calcium chloride solution; and, ferric chloride solution. The feed solution was aerated for 15 to 20 minutes every three or four days. The flow rate was maintained constant at 1.0 mL/minute. The solution was pumped into the base of the column, up through the bed of beads (upflow column). Samples of the influent and effluent were taken and concentrations of DO, TCP, ICl, and pH were measured. The column study was terminated after 45 days to examine the beads with a scanning electron microscope (SEM).

Final Column Study

These experiments were carried out in two cylindrical acrylic columns, and were set up as aerobic, continuous flow packed-bed reactors (Figure 11). The columns had an inside diameter of 5.0 cm and heights of column #1 and #2 were 8.0 cm and 20.0 cm, respectively. Both columns were packed with drained PVA-immobilized cells at a density of approximately 0.9869 g/cm^3. A 5.0 cm diameter 200-sieve mesh copper screen was placed at the top and bottom of each of the columns. A peristaltic pump (Cole-Parmer 7553-30) with two heads (Model 7013) and tygon tubing (ID 0.8 mm, No. 6409-13) was used to pump the groundwater into the columns. The TCP-spiked groundwater was prepared in 25.0 liter glass bottles and covered to prevent photolytic

degradation. Groundwater was spiked with TCP to a final concentration of 10.0 mg/L. Nutrients were added in the amount of 1.0 mL per liter of each of the following solutions previously described: phosphate buffer solution; magnesium sulfate solution; calcium chloride solution; and, ferric chloride solution. The feed solution was aerated for 15 to 20 minutes every three or four days. The flow rate was maintained constant at 1.0 mL/minute. The solution was pumped into the base of the columns, up through the bed of beads (upflow column). Samples of the influent and effluent were taken and concentrations of DO, TCP, ICl, and pH were measured. The column study was monitored for 14 days.

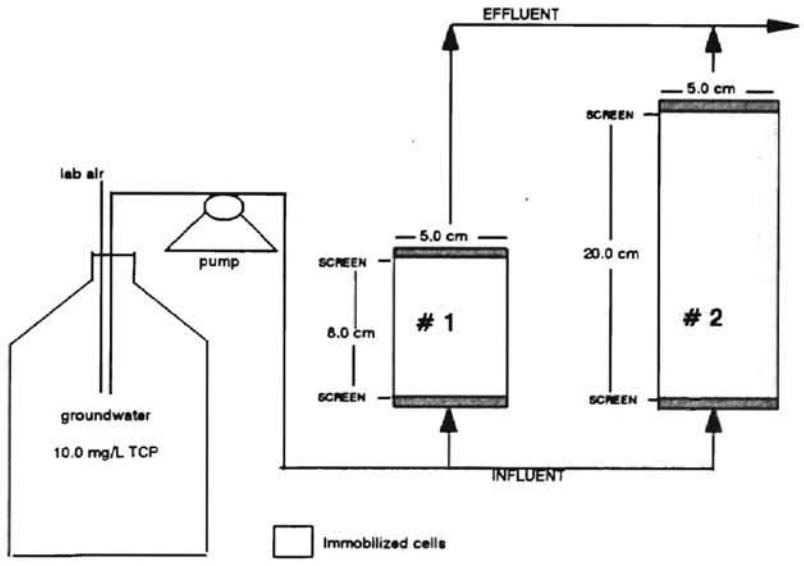


Figure 11. Schematic diagram of final column study.

Tracer Study

Initial Tracer Study

A step tracer study for nonideal flow, as described by Levenspiel (1962), was conducted on the column containing a 10.0 cm bed of beads between layers of aquifer sand used in the the initial column study to predict its flow behavior as a reactor. This technique also gave the column's flowrate and dispersion.

Potassium bromide (KBr) was used as the tracer. Groundwater was added to 148.9 mg of KBr and 1.0 mL of each of the nutrient solutions to make 1.0 liter of bromide tracer solution. The molecular weight of KBr is 119.0 g and Br has a molecular weight of 79.9 g. Br was 67.14% of a mole of KBr. Therefore, 148.9 mg of KBr was needed to obtain 100.0 mg of Br. The measured concentration of Br in the tracer solution was 82.0 mg of Br rather than 100 mg Br. To initiate the tracer study the influent tubing was taken out of the feed bottle and placed into the tracer solution. Samples were taken every 12.0 minutes. Samples were taken for three theoretical hydraulic retention times (HRT), 324 minutes, to ensure enough time for full recovery of the Br concentration. Br concentrations were measured using a Dionex Series 2000i/SP ion chromatograph.

Final Tracer Study

A pulse tracer study for nonideal flow (Levenspiel, 1962) was conducted on columns #1 and #2 used in the final column study. Column #1 contained an 8.0 cm bed of beads and column #2 contained a 20.0 cm bed of beads. The pulse tracer study was

to predict the flow behavior of each column as a reactor. This technique also gave the columns' flow rate and dispersion.

Potassium bromide (KBr) was used as the tracer. Groundwater was added to 297.9 mg of KBr and 1.0 mL of each of the nutrient solutions to make 1.0 liter of 200 mg/L bromide tracer solution. The molecular weight of K is 39.1 g and for Br the molecular weight is 79.9 g. A mole of KBr is 119.0 g. Br is 67.14% of a mole of KBr. Therefore 297.9 mg of KBr was needed to obtain 200.0 mg of Br. To start the tracer study the influent tubing for columns #1 and #2 were taken out of the feed bottle and each placed into 2.0 mLs of the 200 mg/L bromide tracer solution until it was gone. The influent tubing was then placed back into the feed bottle. Samples for column #1 were taken every 4.0 minutes and for column #2 were taken every 10.0 minutes. Samples were taken from each column for three HRTs to ensure that all of the bromide tracer solution was recovered. Samples were taken for 120 minutes for column #1 and 300 minutes for column #2. Br concentrations were measured using a Dionex Series 2000i/SP ion chromatograph.

Analytical Techniques

Multiple extraction methods and gas chromatograph (GC) analytical techniques were used in analyzing the chlorophenol concentrations.

The Voss (1981) method which was modified by Mäkinen (1993) for analysis of small volumes was used to extract chlorophenols from aqueous samples. Samples of 1.0 mL each were acetylated with 25.0 μL of acetic anhydride using 25.0 μL of 5.2 M

K_2CO_3 as the buffer and 25.0 μL of 2,4,6-tribromophenol (5.0 mg/L solution) as the internal standard. The sample was shaken vigorously for 2.0 minutes, the cap was loosened, and then left to stand for 1.0 hour. The acetylated derivatives were extracted by adding 1.0 mL of *n*-hexane. A 1.0 μL extract was used for injection. A five-point (0.1 mg/L, 1.0 mg/L, 5.0 mg/L, 10.0 mg/L, and 20.0 mg/L) standard calibration curve was prepared for the Hewlett-Packard 5890 Series II gas Chromatograph (GC) to analyze TCP by electron capture detector (ECD).

The Hewlett-Packard 5890 Series II GC was equipped with a ^{63}Ni electron capture detector (ECD) and a J&W Scientific fused silica DB-5m column (30 m, 0.32 mm i.d., 0.25 μm film thickness). Carrier gas was helium with a flow rate of 1.3 mL/minute. The oven temperature program was 3.0 minutes at 65°C, ramp at 10°C/minute to 185°C, and hold for 15 minutes at 185°C. The injector temperature was 225°C, and the detector temperature was 250°C.

To identify any breakdown products analyses were performed using a GC-mass spectometry (MS) Hewlett-Packard 5890-5970 MSD. The GC-MS was equipped with a DB-1 capillary column (30 m long, 0.25mm i.d., 0.25 μm film thickness). Helium was the carrier gas with a linear velocity of approximately 40 cm/s. The injection was 1 μL. The injector was at 250°C, and the detector was at 250°C. The oven temperature was 3.0 minutes at 65°C, ramped at 10°C/minute up to 230°C, for a total run time of 20 minutes. The samples were analyzed by Dr. Dilip Sensharma, Mass Spectometry Laboratory, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma.

ENVI-Chrom P SPE tubes (6 mL/250 mg) were used for solid phase extraction

of chlorophenols. The ENVI-Chrom P SPE tubes were obtained from Supelco, Inc., Bellefonte, PA. The resin in the tubes was composed of nonionic, highly crosslinked styrene-divinylbenzene copolymer, the particle size was 8-160 μm, the surface area was 800-950 m^2/g, and the mean pore size was 110-175 Å. The tubes were conditioned sequentially by washing with 6.0 mLs of ethyl acetate, 6 mLs methanol, and 6 mLs of deionized water. A 25 mL sample was added to the tubes with vacuum suction. The tubes were dried for 5 minutes with the vacuum on. The vacuum was turned off and volumetric flasks were placed under the tubes. 2 mLs of ethyl acetate was added to the tube and allowed to wet the packing and soak for 2 minutes. The vacuum was turned on and the tube was washed with an additional 2 mLs and 1 mL of ethyl acetate until 5 mLs of eluant was collected in volumetric flask. Supelco Technical Support Application Note 32 showed ENVI-Chrom P SPE tubes have a mean recovery percent of 103.9 $\pm$ 2.1 for the extraction of TCP. A series of standards were analyzed and a four-point calibration curve (1.0 mg/L; 5.0 mg/L; 10.0 mg/L; and 15.0 mg/L) was developed for the Hewlett-Packard 5890 Series II GC to analyze TCP by flame ionization detector (FID).

The Hewlett-Packard 5890 Series II GC was also equipped with an FID and a Supelco GP 10% SP-2100 on 100/120 Supelcoport 1.8 m X 3.2 mm stainless steel column. The column had a maximum temperature limit of 350°C. The oven temperature setting was isothermal at 200°C. The injection temperature was 275°C and the detection temperature was 275°C. The carrier gas was helium with a flow of 20-30 mLs/minute at 60-80 psi. 2.0 μL of extract was used for injection.

Bromide concentrations from the tracer study were determined with a Dionex Series 2000i/SP ion chromatograph (IC) equipped with an Ionpak AS4A-SC 4mm analytical column and an Ionpak AG4A-SC 4mm guard column. 0.4 mL samples of the effluent were injected into the IC. The IC eluent consisted of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$. A solution of 25 mM H_2SO_4 was used as a column regenerant. A series of standards were analyzed (1.0 mg/L; 2.0 mg/L; 3.0 mg/L; and, 4.0 mg/L) and a four-point calibration curve was developed.

Inorganic chloride ion concentrations were determined by titration using the 408B. Mercuric Nitrate Method from *Standard Methods* (1975).

The pH was measured with a Fisher Scientific Accumet 900 pH meter and probe, model no. 13-620-108.

Dissolved oxygen (DO) was measured with an ORION Research Analog pH meter/model 301 and an ORION model 97-08-00 O_2 electrode.

Volatile suspended solids (VSS) were analyzed by Method 208E. Total Volatile and Fixed Residue at 550°C, *Standard Methods*, 1975.

Electron Micrographs

Scanning electron microscopy samples were prefixed with 1.6% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2.0 hours at room temperature and washed 3 times in the same buffer. The samples were postfixed for 2.0 hours in a buffered 1% (w/v) osmium tetroxide (OsO_4) solution and dehydrated in a graded series of ethanol. The specimens were mounted on aluminum specimen stubs and coated with

gold and palladium. A Jeol JSM 35U scanning electron microscope operating at 25 kV was used for examination of the samples. The samples were prepared and examined by Ginger Baker, Electron Microscopy Lab Manager, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma.

Nuclear Staining and Fluorescence Microscopy

PVA beads containing immobilized cells were fixed in 1.6% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 hours at room temperature. Samples were washed 3 times in same buffer solution. The samples were dehydrated in a graded series of ethanol: 50, 70, 90, 95, 100, 100, 100. The samples were washed three times in propylene oxide. The samples were placed in a 1:1 propylene oxide-spurrs medium overnight at room temperature. Samples were uncapped and placed in a vacuum dessicator for 7 hours and then embedded in 100% spurrs. The samples were placed in an oven at 60°C for 2 days. Samples were cut with a glass knife into thin sections using an RMC MT 6000 at 70 nm and placed on a glass slide, a drop of 2 μg/mL of dissolved 4,6-diamidino-2-phenylindole (DAPI) was added (Shimada et al., 1993). The samples were observed with a NIKON Optiphot-2 epifluorescence microscope. The thin sections of the samples were prepared by Ginger Baker, Electron Microscopy Lab Manager, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma. The samples were stained with DAPI and examined by Dr. David H. Demezas, Department of Microbiology, Oklahoma State University, Stillwater, Oklahoma.

Summary

Table 2 summarizes the studies and experiments conducted, specific objectives for each study, information expected from each study, and how that information relates to the evaluation of permeable barrier technology using PVA-immobilized cells.

TABLE 2.

SUMMARY OF EXPERIMENTS/STUDIES

Experiment/ Study	Specific Objectives	Expected Information	How expected results relate to evaluation of permeable barrier technology
(1) Porosity test	*Measure* the ability or capacity of the packed bed of beads to take up groundwater into its pore spaces.	▪ specific gravity ▪ density ▪ porosity percent	The porosity percent is needed to determine if the porosity percent is similar or compatible to the aquifer material so that the flow of groundwater would not be impeded by a less porous material.
(2) Falling head permeameter test	*Measure* the ability of the packed bed of beads to transmit groundwater.	▪ permeable coefficient (k).	Permeability is needed to determine if the groundwater will flow through the bed of beads and if at a compatible rate to that of the aquifer to avoid the bed of beads acting as a dam.
(3) Compression test	*Measure* the amount a packed bed of beads compresses as load is increased.	▪ deformation percent ▪ compressibility index (C_c)	The load applied is equivalent to the weight of a saturated bed of beads at 40 feet in depth. This will indicate if the beads are strong enough at the bottom of the ditch or if the deformation will effect its performance as a permeable barrier medium.
(4) Diffusion study	*Measure* the rate at which TCP diffuses into PVA beads.	▪ diffusion coefficient (D)	The diffusion rate is needed to know how fast the TCP molecules diffuse into the PVA bead to get substrate to the entrapped organisms.
(5) Adsorption study	*Measure* the amount of TCP that is adsorbed onto the copper screen and onto the PVA beads.	▪ adsorption capacity (K) ▪ adsorption intensity (1/n)	It is important to know amount of TCP that is physically removed so that any other removal will be contributed to biological degradation.

TABLE 2 - *Continued.*

Experiment/ Study	Specific Objectives	Expected Information	How expected results relate to evaluation of permeable barrier technology
(6) Kinetic study	*Measure* the amount of TCP degraded, DO consumed, and ICl released by the free cells and the immobilized cells.	▪ substrate utilization rates (r_{su}) of the free cells and the immobilized cells ▪ growth rate (r_g) of the free cells.	The batch study will show that the free cells are active, growing, and utilizing the TCP as substrate prior to immobilizing them. The immobilized cells will show that they too are capable of utilizing TCP as a substrate. The ICl increases tend to confirm that dehalogenation of TCP is occurring. DO consumption will verify that it is an aerobic system.
(7) Column study	*Simulate* a "bio-trench" using PVA immobilized cells as a permeable barrier media	▪ biodegradation rate of TCP by the continuous flow reactor ▪ effect of biodegradation rate by different hydraulic retention times	The column study will show the applicability of PVA immobilized cells as a permeable barrier medium and its capability as a biological carrier for TCP degradation.
(8) Tracer study	*Measure* the rate at which the feed solution travels through the columns and the residence time in the columns.	▪ flowrate (Q) ▪ reactor dispersion number ($D/\mu L$)	The tracer study will show the flow behavior and amount of time that the feed solution is in contact with the immobilized cells for degradation of TCP.

TABLE 2 - *Continued.*

Experiment/ Study	Specific Objectives	Expected Information	How expected results relate to evaluation of permeable barrier technology
(9) DAPI stain	*Verify* that organisms were immobilized	▪ Fluorescence of the organisms under an epifluorescence microscope	Will verify that organisms were immobilized prior to setting up column study.
(10) Electron micrographs	*Verify* that organisms were immobilized and to show any changes that occurred over time.	▪ bacteria population ▪ immobilization sites ▪ morphology ▪ colonies ▪ physical changes	Verifies that organisms were immobilized. It will show where the organisms are immobilized within the bead. It will show the morphology and any colonization of the organisms. The micrograph will show physical changes of the beads over time.
(11) GC-MS	*Identify* chlorinated compounds and *support* dehalogenation of TCP.	▪ analysis of chlorinated compounds	Will identify TCP and any intermediate compounds that may develop as TCP degrades. These results will tend to support dehalogenation of TCP.

CHAPTER IV

RESULTS AND DISCUSSIONS

Experiments were conducted as part of this feasibility study to test PVA-immobilized cells for the purpose of being used as a permeable barrier media. Two aspects of using the PVA-immobilized cells as a permeable barrier medium were assessed: 1) physical characteristics of the PVA beads; and, 2) ability as a biological carrier system. An examination of the physical characteristics of the beads included preparing blank beads with different molecular weights of PVA. The beads were evaluated and compared as to ease of production, elasticity, and firmness. Porosity, permeability, and compressibility of a packed bed of beads were the physical parameters evaluated for applicability as a medium in a permeable barrier. Batch studies were conducted to measure adsorption, diffusion, and substrate utilization. Adsorption studies of the beads were studied to determine if any physical removal of TCP was occurring. Diffusion studies were conducted to evaluate the rate of TCP diffusion into the PVA beads. Kinetic studies were conducted to determine the substrate utilization rate of free cells and immobilized cells and the growth rate of free cells. Column studies were conducted to simulate the concept of PVA-immobilized cells being used as a biological permeable barrier system.

Beads Prepared with Different Molecular Weights of PVA

Beads prepared with different molecular weights (MW) of PVA (MW 88,000; MW 115,000; and, MW 126,000) were evaluated qualitatively as to ease of production, elasticity, and firmness. The beads prepared with PVA (MW 126,000) formed a very viscous solution that was difficult to extrude through the tygon tubing (ID 3.1 mm). The beads were too firm, almost hard, and were not very elastic. Beads prepared with PVA (MW 115,000) also formed a very viscous solution that was difficult to extrude. The beads were very firm and not very elastic. Beads prepared with PVA (MW 88,000) formed a solution that could easily be extruded through tygon tubing (ID 3.1 mm). Beads were rubber-like, and elastic. The qualitative observations made and the results of the evaluation are shown in Table 3.

TABLE 3.

COMPARISON OF BEADS PREPARED WITH DIFFERENT MOLECULAR WEIGHTS OF POLYVINYL ALCOHOL (PVA)

MW (g)	Ease of Making	Elasticity	Firmness	Uniformity	Bead size (mm)
126,000	difficult, too thick, very viscous	not very elastic	hard	good	3 - 5
115,000	difficult, too thick, very viscous	not very elastic	too firm	good	3 - 5
88,000	easily extruded	rubber-like and elastic	firm	excellent	3 - 5

PVA (MW 88,000) was chosen for bead preparation for the adsorption and diffusion studies and for cell immobilization because of its ease in making beads and its rubber-like and elastic properties. A batch of beads made with 43.7 g PVA (MW 88,000) as described previously produced a 350 mL volume of beads which contained approximately 4272 beads with diameters of 3 mm to 5 mm (Figure 12).

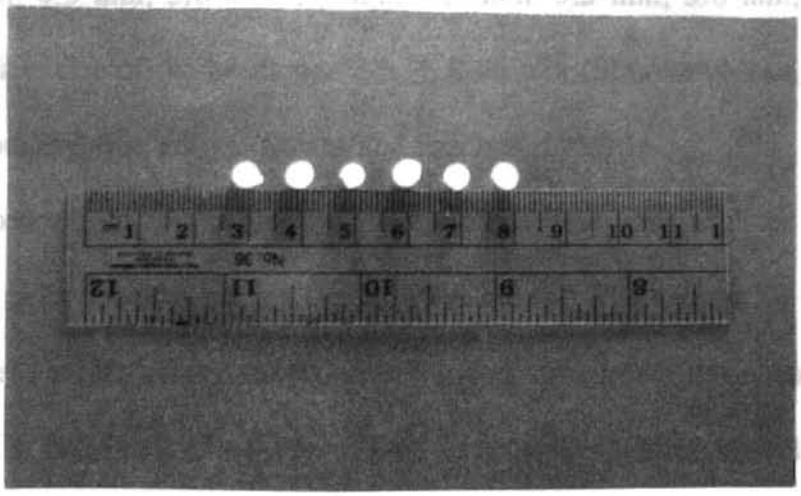


Figure 12. Bead size (3mm-5mm).

Characterization of PVA Beads

The results of the characterization of a packed bed of PVA beads are shown in Table 4 and Figures 13 and 14. The pH of the saturated beads in groundwater was taken prior to setting up the columns. The pH of the saturated beads was 8.1. The density of drained beads at 22°C was 0.9869 g/cm^3 and density of water was 0.9793 g/cm^3. The bead density was slightly greater than water which would eliminate problems with water-

saturated beads floating. The measured porosity at 22°C for the drained beads was 25%. The porosity of the beads is comparable to average porosities of aquifer materials composed of gravel and sand (Linsley, Jr. et al., 1982). The permeability coefficient (K) obtained from the falling head permeameter test for the beads at 22°C was 0.1425 cm/s. This permeability coefficient (K) is comparable to typical permeability values found for coarse sand to fine gravel (Smith, 1979). Ten beads used in the permeameter test were selected at random. The diameter of each bead was measured as follows: 3.7 mm, 3.3 mm, 4.5 mm, 4.3 mm, 3.8 mm, 3.9 mm, 4.1 mm, 3.5 mm, 3.8 mm, 3.6 mm. The average particle size of the beads was 3.8 $\pm$ 0.36 mm (SD) which can be classified as a uniform rounded fine gravel (Means and Parcher, 1963).

An oedometer (or consolidation test apparatus) was used to determine the compression behavior of a packed bed of PVA beads. The amount of compression in inches was read directly from the oedometer guage. The stress, time, and consolidation are recorded in Table 9, Appendix B. Figure 13 shows the percent cumulative strain that occurs at different depths and predicted pressures. The bed of beads compressed 48% with an overburden pressure equivalent to that found at the bottom of a 40 ft ditch. The curve shows that a rapid increase in strain was followed by a slower rate for the remaining test. This means there was a significant amount of compression relatively quickly at shallow depths and less compression as depth increased. The compressibility index (C_c) is a standard measurement used in soil mechanics to determine the volumetric strain of a soil per the unit pressure applied. The compressibility index (C_c) of a saturated bed of PVA beads was determined to be 4.08 X 10^{-3} m^2/kN which is

comparable to a soft clay (Smith, 1979). Figure 14 shows the consolidation of a bed of beads over time (Means and Parcher, 1963). The greatest amount of consolidation, 0.170 inches, took place early on in the test as stress was increased to 1.28 tons/ft^2 over a 107 minute period. As stress was held constant at 1.28 tons/ft^2 the bed of beads consolidated an additional 0.019 inch over a 400 minute period. Therefore, 89% of the total consolidation of 0.189 inches took place during the first 107 minutes as stress was increasing and only 11% during the last 400 minutes as stress was held constant. The beads had compressed into a cake that adhered together when removed from the oedometer cell. The beads were placed in water and within 24 hours had expanded to their original volume.

Characterization of Aquifer Sand

The results of the characterization of a packed bed of aquifer sand is shown in Table 4. The pH of the saturated sand was taken prior to setting up the column in the initial column study. The pH of the saturated sand was 6.4 to 6.9. The air dried sand was packed into the column with a density of 1.6 g/cm^3 to simulate actual densities found in alluvial aquifers (Mandel and Shiftan, 1981). The measured porosity at 22°C for the sand was 30%. The porosity of the sand was comparable to an average porosity of aquifer sand (Linsley, Jr., et al., 1982). The permeability coefficient obtained from the permeameter test for the sand at 22°C and density of 1.6 g/cm^3 was 0.0162 cm/s. The permeability coefficient was comparable to a medium grained sand (Smith, 1979). The sand was sieved and the average particle size was 0.4 mm (40 mesh size) and can be

classified as a uniform rounded medium sand (Means and Parcher, 1963). The compressibility index (C_c) value of 2.87 X 10^{-5} m^2/kN for the sand was obtained from Smith (1979).

TABLE 4.

CHARACTERISTICS OF PVA BEADS AND AQUIFER SAND

Parameters	Packed Bed PVA Beads	Packed Bed Aquifer Sand
pH	8.1	6.4-6.9
Specific Gravity*	1.008	1.634
Density* (ρ) (g/cm^3)	0.9869	1.6
Porosity (%)	25	30
Permeability Coefficient (K) (cm/s)	0.1425	0.0162
Compressibility Index (C_c) (m^2/kN)	4.08 X 10^{-3}	2.87 X 10^{-5}
Particle Size (mm)	3.8	.4
Soil Classification	uniform rounded fine gravel	uniform rounded medium sand

*Density of water at 22°C was 0.9793 g/cm^3.

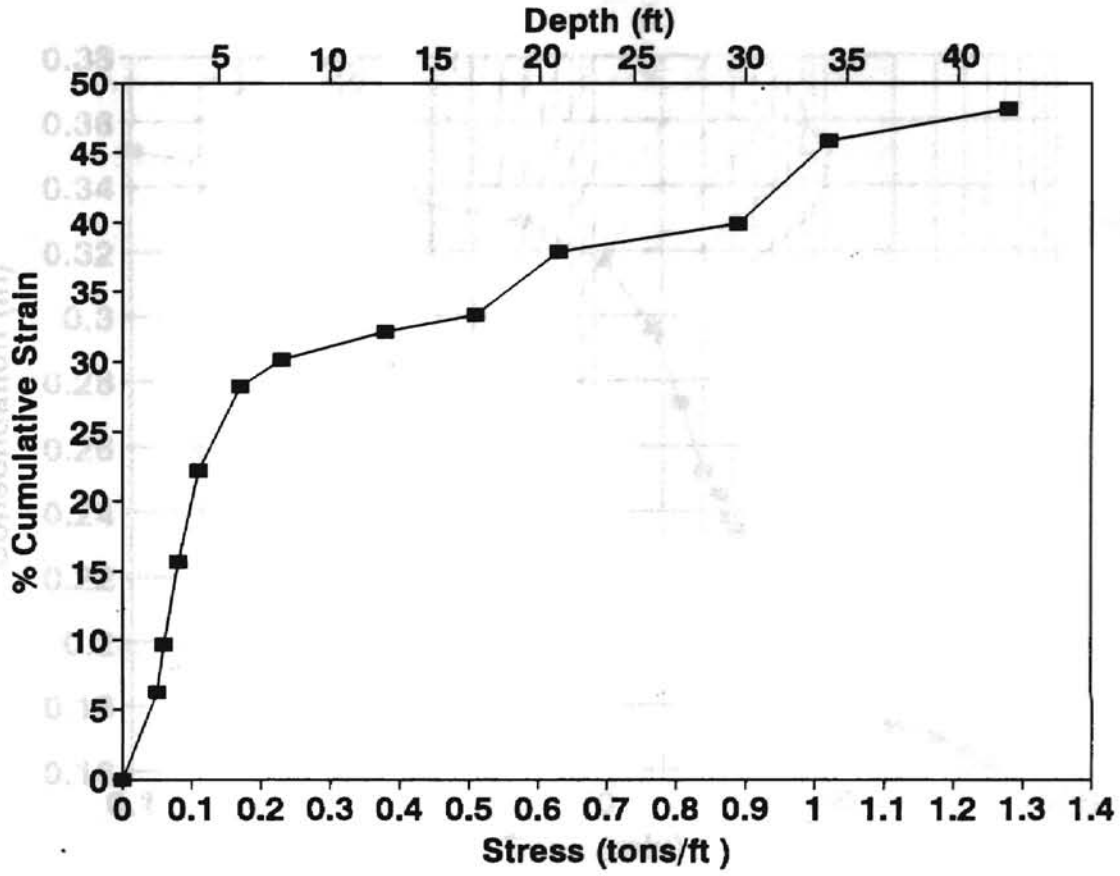


Figure 13. Compressibility of PVA Beads.

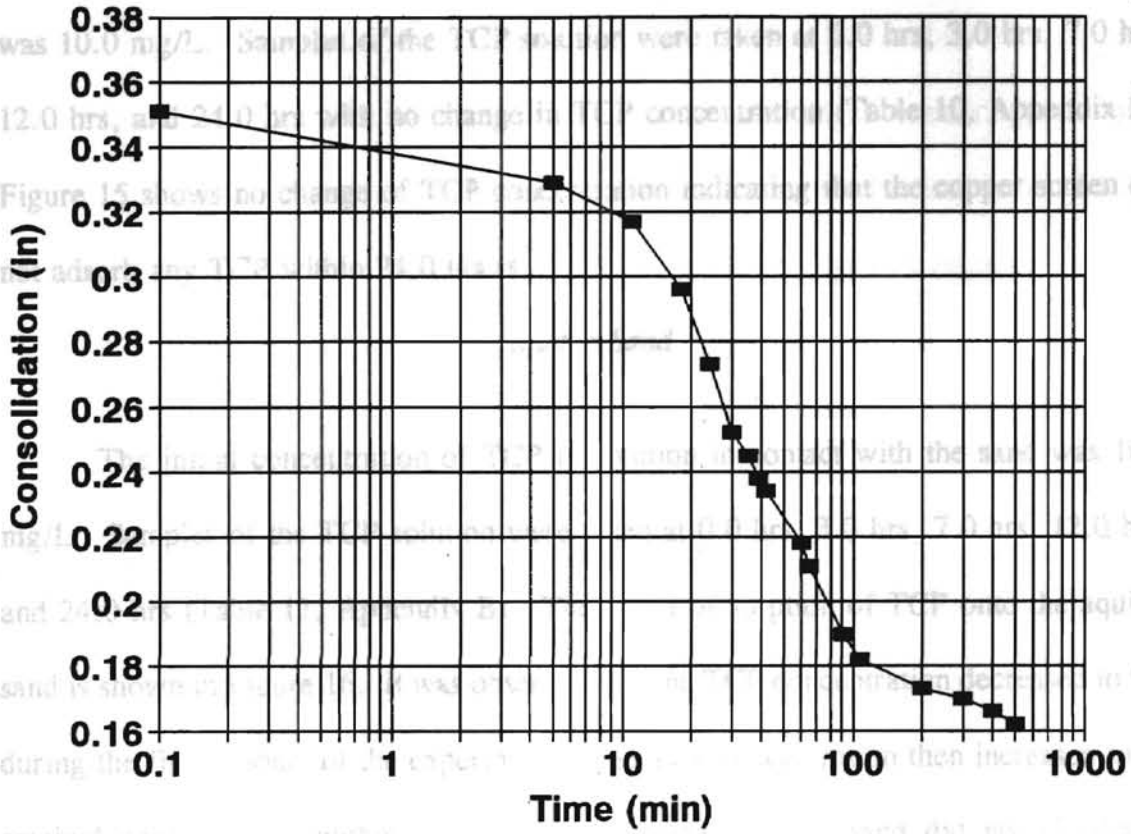


Figure 14. Consolidation of PVA Beads.

Batch Studies

Adsorption Studies

Copper Screen

The initial concentration of TCP in the solution in contact with the copper screen was 10.0 mg/L. Samples of the TCP solution were taken at 0.0 hrs, 3.0 hrs, 7.0 hrs, 12.0 hrs, and 24.0 hrs with no change in TCP concentration (Table 10, Appendix B). Figure 15 shows no change of TCP concentration indicating that the copper screen did not adsorb any TCP within 24.0 hours.

Aquifer Sand

The initial concentration of TCP in solution in contact with the sand was 10.0 mg/L. Samples of the TCP solution were taken at 0.0 hrs, 3.0 hrs, 7.0 hrs, 12.0 hrs, and 24.0 hrs (Table 11, Appendix B). The effect of sorption of TCP onto the aquifer sand is shown in Figure 16. It was observed that the TCP concentration decreased to 9.6 during the first 3 hours of the experiment. The TCP concentration then increased to its original concentration within 24.0 hours indicating that the sand did not physically remove any TCP.

PVA Beads

An initial equilibrium study was conducted on 50.0 g of blank PVA beads in a continuously stirred 500 mL volume of 20.0 mg/L TCP solution. Samples of the solution were taken at various times during a 24 hour period to analyze TCP concentrations (Table 12, Appendix B, and Figure 17). It was observed that the TCP concentration reached an equilibrium concentration of 14.5 mg/L within 3.0 hours of the

experiment. As a result of the equilibrium test the adsorption study was conducted for 24.0 hours. This study consisted of adding varying masses of drained beads (0.0 g, 1.0 g, 5.0 g, 10.0 g, 15.0 g, and 20.0 g) to 5 glass flasks of 250 mL volumes. A 100 mL volume of a 24.0 mg/L TCP solution was poured into each flask. Flasks were covered to prevent photolytic degradation and were shaken for 24 hours. The data from the batch study (Table 13, Appendix B) were plotted and the resulting Freundlich isotherm is shown in Figure 18. Data were also plotted on other isotherms, but the Freundlich had the best correlation. The following linearized form of the Freundlich equation was used to evaluate adsorption capacity (Sawyer et al., 1994):

$$Log\ q = Log\ K + \frac{1}{n}\ Log\ C \qquad (5)$$

Where
q = X/M (mg/g)
X = amount of adsorbate (mg/L)
M = mass of adsorbent at equilibrium (g)
C = liquid-phase concentration (mg/L)
K = measure of adsorption capacity (L/g)
1/n = adsorption intensity

A regression analysis of the data resulted in a linear fit within an r-squared of 0.96, a slope of 1/n and an intercept Log K at Log C = 0 (C=1). The slope (1/n) was determined to be 11.1. The intercept Log K was determined to be -14.3 and by computing the antilog the K value was 5.01 X 10^{-15} (L/g). Figure 18 shows that for a 10.0 mg/L TCP solution and a 20.0 mg/L TCP solution the q = X/M values are 6.31 X 10^{-4} mg/g and 1.5 mg/g, respectively. As the TCP concentration increased in solution the adsorption capacity of the PVA beads also increased. Therefore, minimal adsorption occurred from the 10.0 mg/L TCP solution used in the batch and column studies.

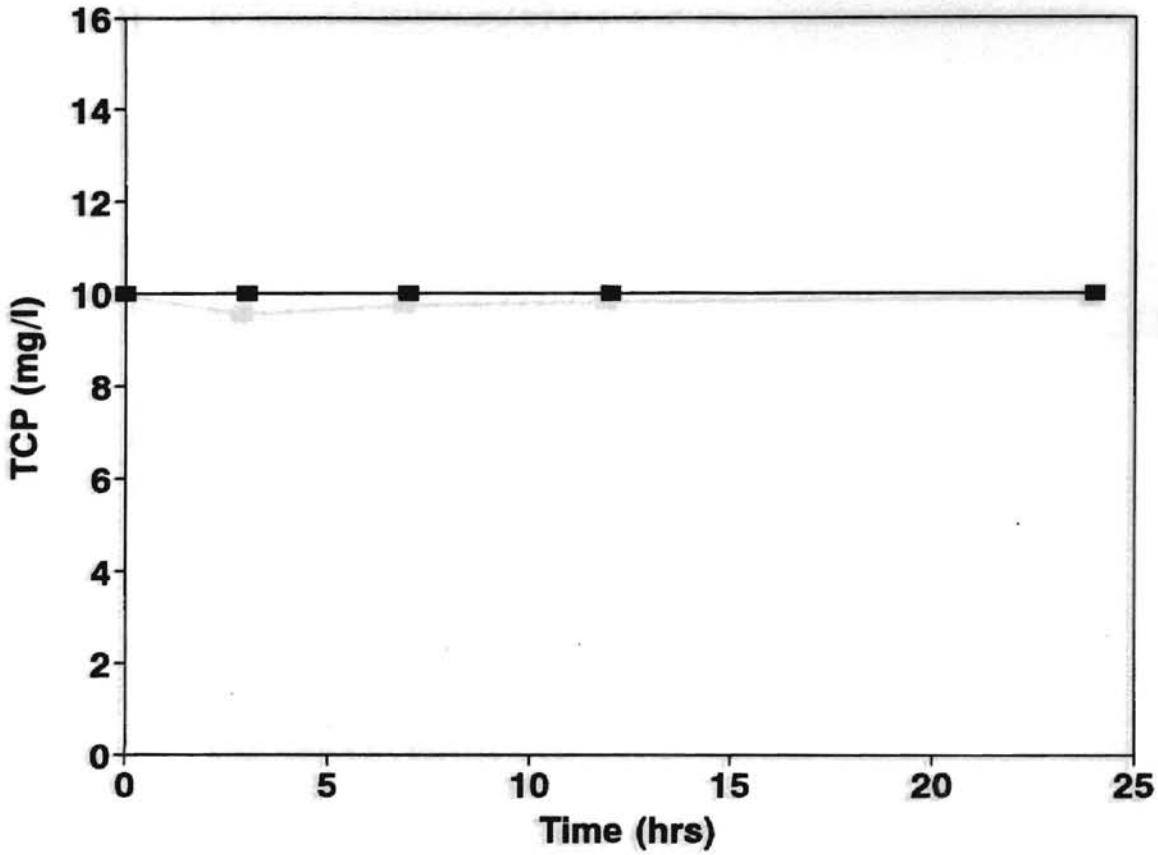


Figure 15. Sorption of TCP onto Copper Screen.

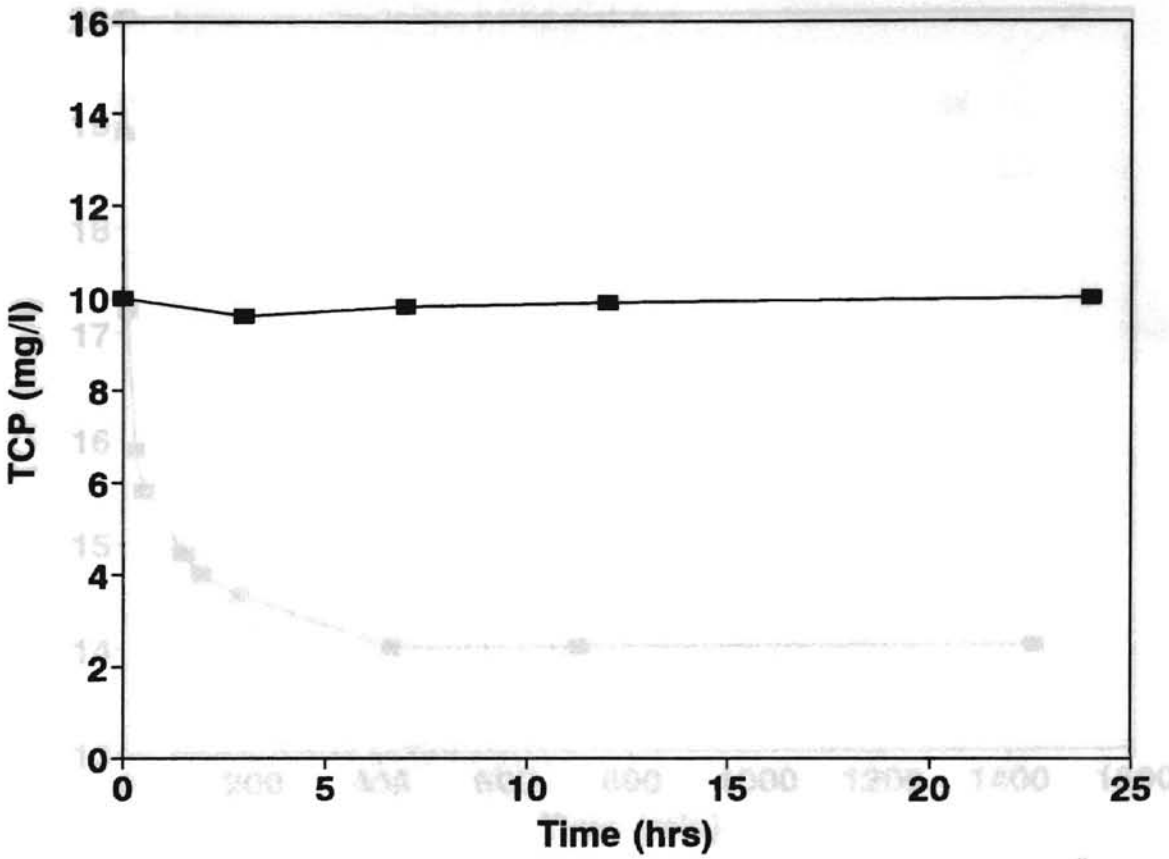


Figure 16. Sorption of TCP onto Aquifer Sand.

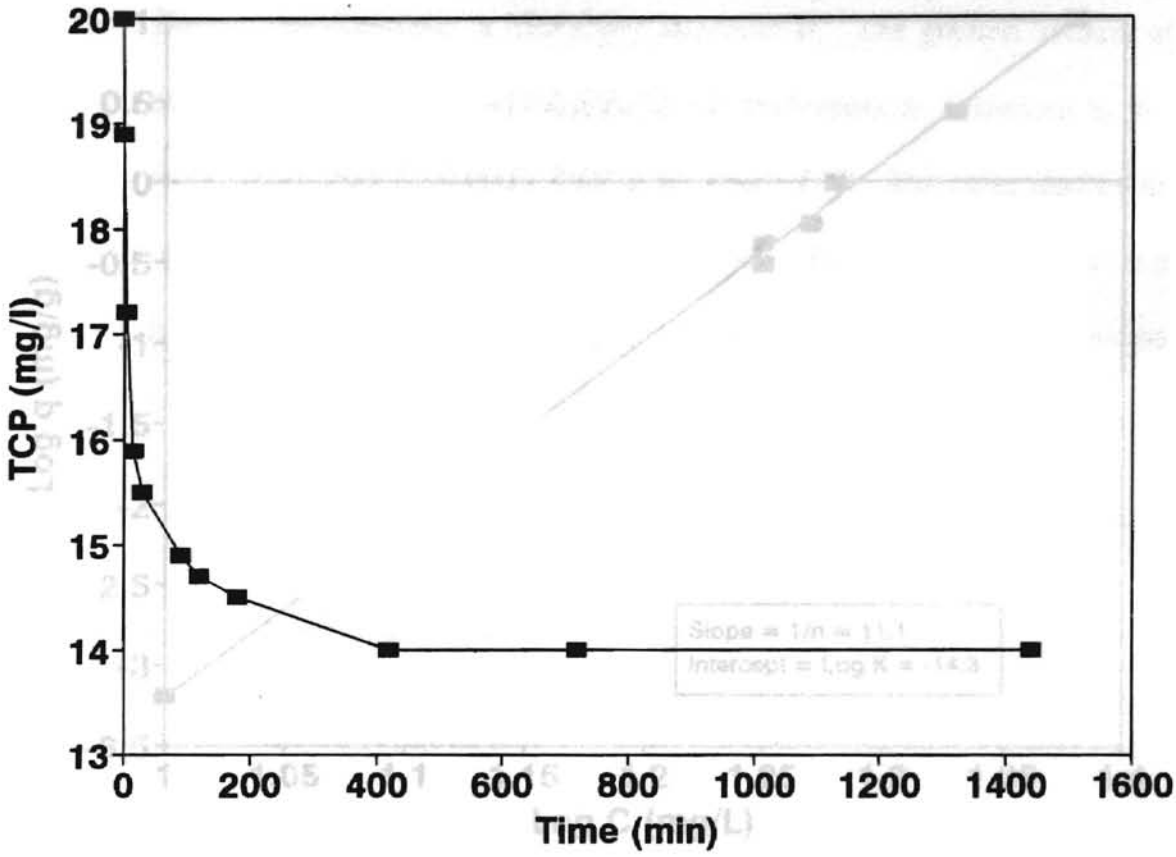


Figure 17. Equilibrium Study for PVA Beads and TCP.

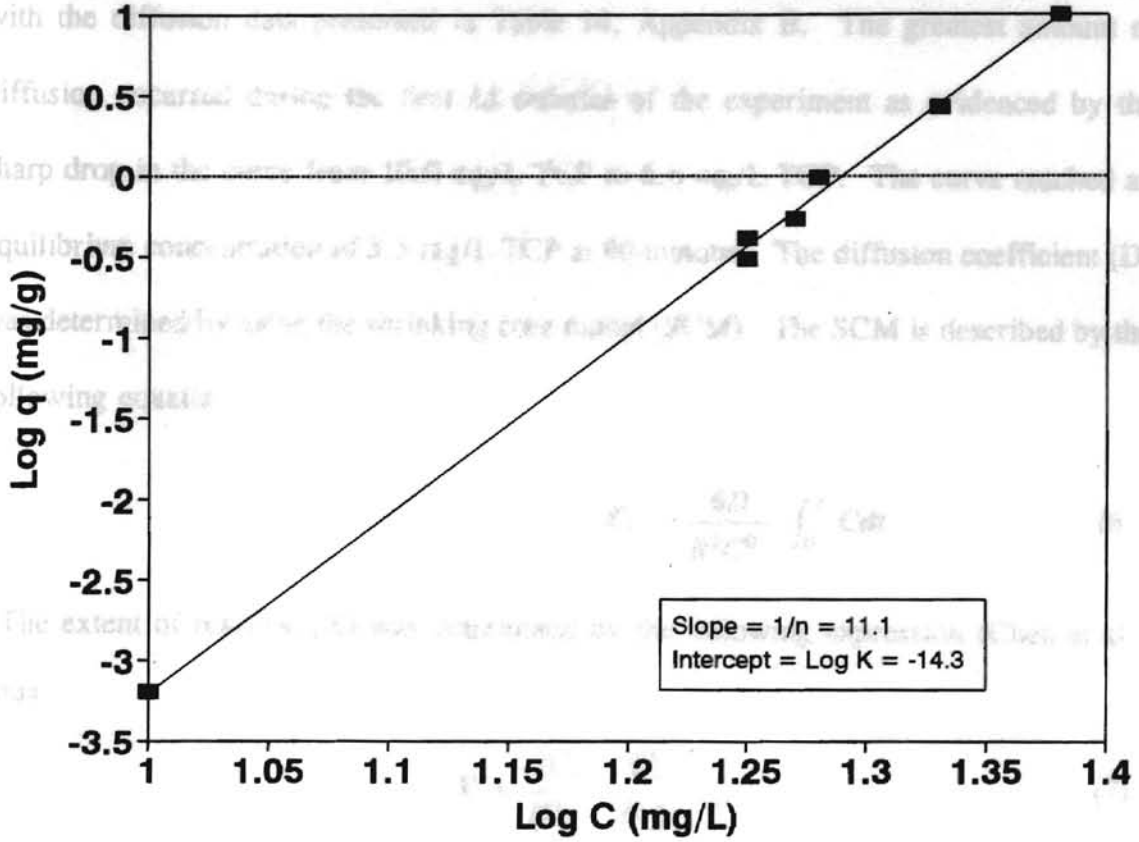


Figure 18. Freundlich Isotherm for Adsorption of TCP onto PVA Beads.

Diffusion Study

The diffusion of TCP from solution into blank PVA beads is shown in Figure 19 with the diffusion data presented in Table 14, Appendix B. The greatest amount of diffusion occurred during the first 45 minutes of the experiment as evidenced by the sharp drop in the curve from 10.0 mg/L TCP to 6.6 mg/L TCP. The curve reached an equilibrium concentration of 5.5 mg/L TCP at 90 minutes. The diffusion coefficient (D) was determined by using the shrinking core model (SCM). The SCM is described by the following equation (Chen et al., 1993):

$$1 - 3(1 - X)^{2/3} + 2(1 - X) = \frac{6D}{R^2 C^0} \int_0^t Cdt \qquad (6)$$

The extent of reaction (X) was determined by the following expression (Chen et al., 1993):

$$X = \frac{[C_0 - C]}{[C_0 - C_\infty]} \qquad (7)$$

The average binding site density of PVA (C^0) was determined by the following expression (Jang, 1994):

$$C^0 = [C_0 - C_\infty] \left[\frac{volume\ of\ reactor}{volume\ of\ spheres}\right] \qquad (8)$$

The values for $F(X) = 1 - 3(1-X)^{2/3} + 2(1 - X)$ were calculated (Exhibit C) and are presented in Table 15, Appendix B. The integration of $\int Cdt$ was evaluated by the trapezoid rule and calculations are shown in Exhibit C. Determined values are presented in Table 15, Appendix B. The slope was obtained from the curve of $F(X) = 1 - 3(1-$

$X)^{2/3} + 2(1 - X)$ vs $\int Cdt$ (Figure 20). A regression analysis of the data resulted in a linear fit within an r-squared of 0.94.

Diffusivity (D) was determined from the following equation (Chen et al., 1993):

$$D = \frac{[Slope]C^0 R^2}{6} \quad (9)$$

Where

C = concentration of TCP in solution at a given time (mg/L)
C_0 = initial concentration of TCP in solution (mg/L)
C_∞ = concentration of TCP in solution at equilibrium (mg/L)
C^0 = average binding site density of PVA (mg/L)
D = diffusion coefficient (cm^2/s)
R = radius of bead (cm)
t = time (s)
X = extent of reaction

The average binding site density of PVA was determined from Equation (8) to be 32.1 mg/L (Appendix C). The concentration of TCP in solution at equilibrium (C_∞) was 5.5 mg/L. The volume of the reactor was 500 mLs and the volume of spheres was 70 mLs. The slope of the curve of $F(X) = 1 - 3(1-X)^{2/3} + 2(1 - X)$ vs $\int Cdt$ was determined to be 0.9625×10^{-3} L/mg·min. The average radius (R) of the beads was 0.19 cm. The D coefficient was determined from Equation (9) to be 3.1×10^{-6} cm^2/s (Appendix C). This was similar in comparison to rates of diffusion into other bead materials. Jang (1994) reported a similar D coefficient of 1.18×10^{-5} cm^2/s for diffusivity of Cu^{2+} into 2% alginate beads. Tanaka (1984) reported a D coefficient of 6.8×10^{-6} cm^2/s for diffusivity of glucose into 2% Ca-alginate gel beads.

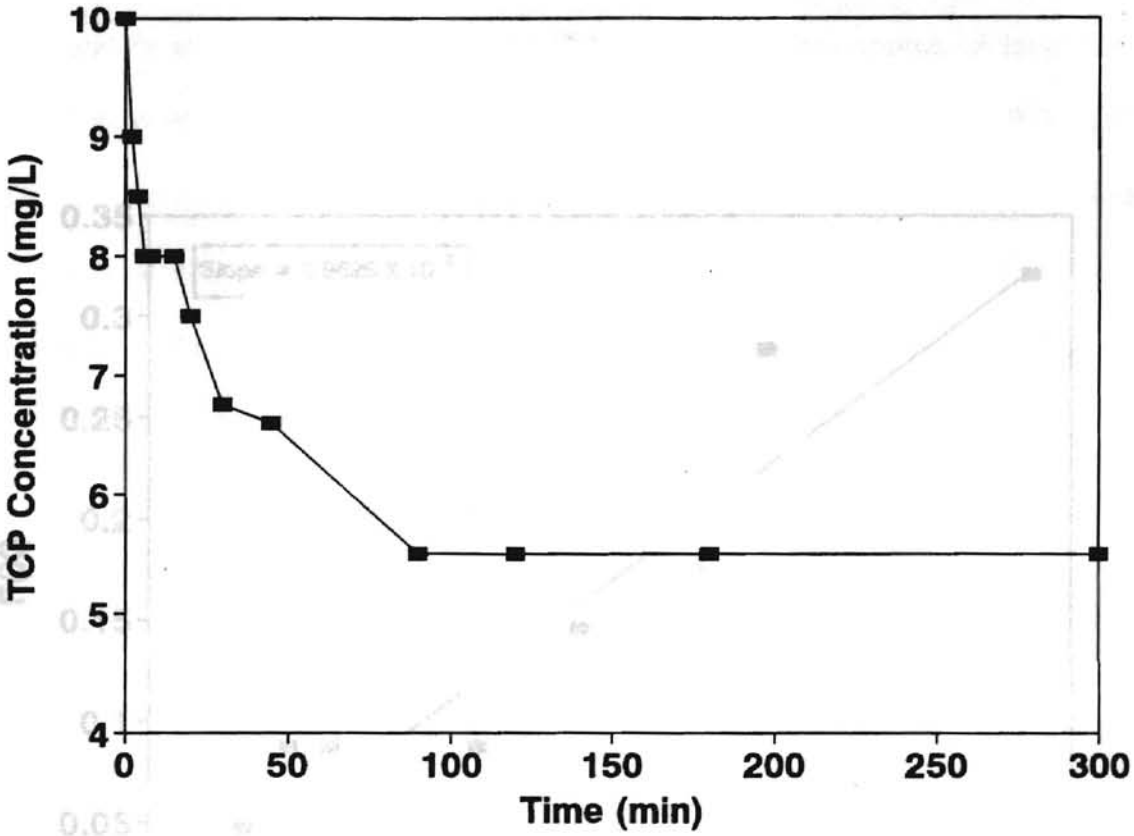


Figure 19. Diffusion of TCP into PVA Beads.

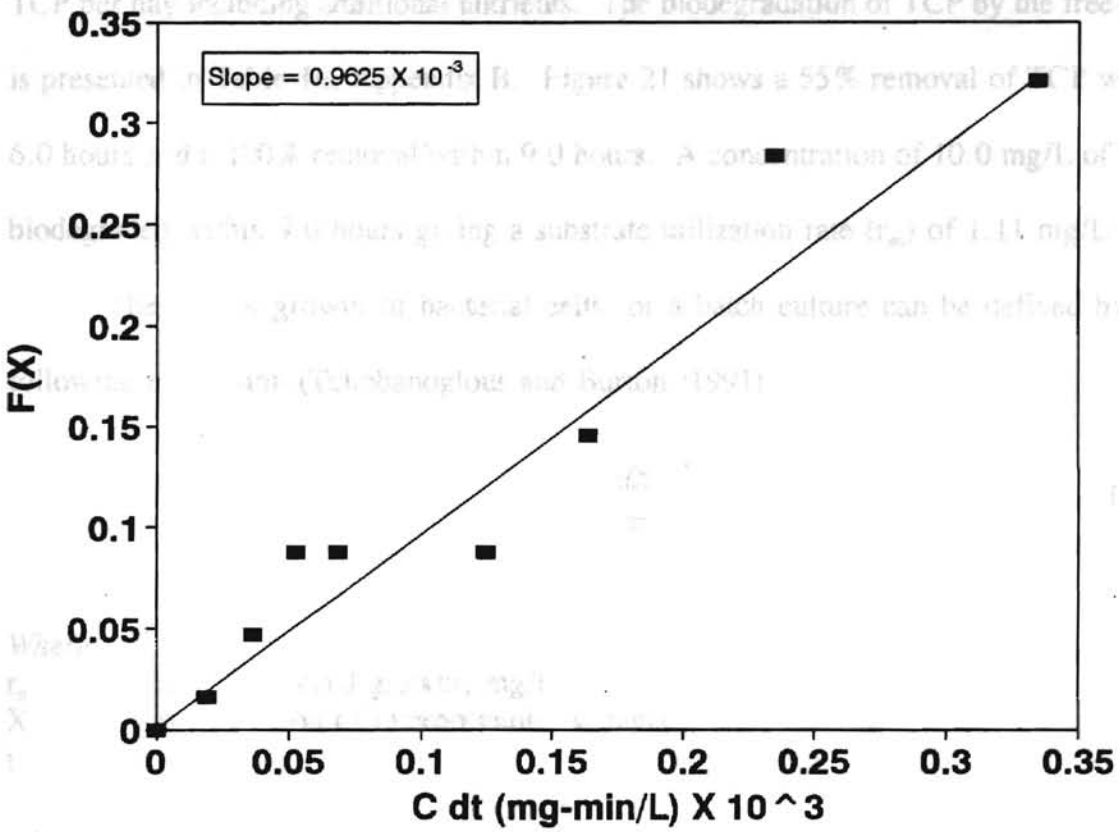


Figure 20. Shrinking Core Model - Diffusion Coefficient

Kinetic Studies

Free Cells - Biodegradation of TCP

This batch experiment was conducted to determine the growth rate of the free cells and the substrate utilization rate for TCP as the sole carbon source. A batch culture of the activated sludge obtained from the pulp mill was aerated and fed 10.0 mg/L of TCP per day including additional nutrients. The biodegradation of TCP by the free cells is presented in Table 16, Appendix B. Figure 21 shows a 55% removal of TCP within 6.0 hours and a 100% removal within 9.0 hours. A concentration of 10.0 mg/L of TCP biodegraded within 9.0 hours giving a substrate utilization rate (r_{su}) of 1.11 mg/L· hr.

The rate of growth of bacterial cells for a batch culture can be defined by the following expression (Tchobanoglous and Burton, 1991):

$$r_g = \frac{dX}{dt} \qquad (10)$$

Where

r_g = rate of bacterial growth, mg/L·hr
X = concentration of microorganisms, mg/L
t = time, hr

The results of the VSS analysis are presented in Table 16, Appendix B. The VSS increased from 888 mg VSS/L to 923 mg VSS/L within 9.0 hours. From Equation 10 this gave a growth rate (r_g) of 3.9 mg VSS/L· hr.

The following stoichiometric equation for aerobic mineralization of TCP shows

that 3 moles of HCl are produced per mole of TCP (Mäkinen, et al., 1993).

$$C_6Cl_3H_2OH + 5.5O_2 \rightarrow 6CO_2 + 3HCl \quad (11)$$

The theoretical increase of inorganic chlorides was predicted to be 5.39 mg/L for dehalogenation of 10.0 mg/L of TCP as follows:

$$\frac{10\ mg/L\ TCP}{197.5\ g/mole\ TCP}\left(\frac{3\ moles\ Cl^-}{mole\ TCP}\right)35.5\ g/mole\ Cl^- = 5.39\ mg/L\ Cl^- \quad (12)$$

Theoretical inorganic chloride releases based on TCP concentrations were predicted and are presented in Table 16, Appendix B. Measured inorganic chloride releases had an overall increase of 8.0 mg/L (550 mg/L - 558 mg/L) over a period of 9.0 hours in which 10.0 mg/L was removed. The 8.0 mg/L ICl increase tends to confirm dehalogenation of TCP. Measured ICl data are presented in Table 16, Appendix B. Theoretical and measured ICl releases are compared in Figure 22. The theoretical and the measured inorganic chloride increases were very close in value, but with measured values being slightly higher. These small differences in value could result from human error.

Influent samples (groundwater with 10.0 mg/L TCP and added nutrients) were spiked with known Cl^- concentrations of 0.0 mg/L, 3.0 mg/L, 5.0 mg/L, and 7.0 mg/L. Each sample was analyzed using the 408B. Mercuric Nitrate Method from *Standard Methods* (1975). The total concentrations of Cl^- for each sample were determined to be 162 mg/L, 165 mg/L, 167 mg/L, and 169 mg/L, respectively. The results showed that an increase as low as 3.0 mg/L Cl^- was detected using this method of analysis.

Immobilized Cells - Biodegradation of TCP

This batch experiment was conducted to determine the substrate utilization rate of TCP as the sole carbon source. The wet centrifuged biomass used in this experiment for immobilization weighed 7.2 grams. The VSS of the centrifuged biomass was approximately 259 mg. This approximation was made from a previous analysis where 0.1905 g VSS was determined from 5.2805 g of centrifuged biomass (0.036 g VSS/g centrifuged biomass). Therefore, approximately 259 mg VSS was immobilized in PVA beads. The biodegradation of TCP by the immobilized cells is presented in Table 17, Appendix B. The experiment was done three times in a series. The removal rate was progressively faster for each subsequent experiment. Figure 23 shows that it took 72 hours after the cells were first immobilized for 100% removal. The second time it took 24 hours for 100% removal and the third time it took 5.0 hrs for complete removal of TCP.

Different methods to determine the rate of growth of the bacteria were researched and evaluated. Such methods considered were optical density, weighing the beads, and measurement of protein. These methods were not used because they could not be reliably applied to bacteria immobilized in PVA. The activated sludge used in this study was a mixed bioculture containing particles of sand and grit which would distort optical density results. Weighing the beads to determine any increase in mass which could be attributed to bacterial growth was considered as a viable method. The potential problem with this method was accuracy because of the extremely small weight changes. Measuring the protein was another method considered, but the PVA beads could not be

dissolved without destroying the bacteria. Therefore the rate of growth of bacteria inside the beads was not determined.

The substrate utilization rates (r_{su}) were determined for the first, second, and third experiments to be 0.139 mg/L· hr, 0.417 mg/L· hr, and 2.0 mg/L· hr, respectively. The r_{su} was progressively faster for each subsequent experiment which suggested that the immobilized cells needed time to overcome the effects of immobilization, such as low pH conditions. The DO and ICl releases were measured during the third experiment.

Inorganic chloride releases were measured and are presented in Table 18, Appendix B. Figure 24 shows the theoretical increase in ICl that was determined for the dehalogenation of 10.0 mg/L TCP as compared to the measured increases in ICl from the immobilized cells. The measured ICl value of 7.0 mg/L at 5.0 hours for the complete dehalogenation of 10.0 mg/L TCP was slightly higher than the 5.39 mg/L theoretical *ICl* value. The measured increases in ICl tend to confirm that complete dehalogenation of TCP occurred.

Theoretical oxygen consumption was predicted from Equation (11) to be 0.89 mg/L oxygen per 1.0 mg/L TCP biodegraded. To biodegrade 10.0 mg/L TCP approximately 8.9 mg/L oxygen would need to be consumed. There was a 2.6 mg/L decrease in dissolved oxygen (DO) (8.6 mg/L - 6.0 mg/L) over a period of 5.0 hours. The DO consumption of 2.6 mg/L gave evidence of aerobic activity occurring, but too low to prove complete aerobic biodegradation of 10.0 mg/L TCP.

This batch culture of immobilized cells was continued for 45 days by feeding it 10.0 mg/L of TCP, including additional nutrients daily. Samples of the TCP solution

were collected on the 45th day of operation. Samples were extracted using the Voss (1981) method as previously described. The Voss extraction method produced acetylated derivatives of the original chlorinated compounds. The samples were analyzed by GC-ECD which showed an initial TCP concentration of 10.0 mg/L at 0.0 hours and a final TCP concentration of 0.0 mg/L at 5.0 hours. The same samples were analyzed by GC-MS and the resulting chromatographs for the samples at 0.0 hours and 5.0 hours are shown in Figures 25 and 26, respectively. The chromatograph in Figure 25 for the sample at 0.0 hours, with a known concentration of 10.0 mg/L TCP, shows a peak at 11.1 minutes. A mass spectrum scan was completed on this peak and it was identified as 2,4,6-trichlorophenyl acetate. 2,4,6-trichlorophenyl acetate was the acetylated derivative of 2,4,6-TCP. The chromatograph in Figure 26 for the sample taken at 5.0 hours shows no peaks for TCP or any intermediate chlorinated compounds which further supports the complete dehalogenation of 10.0 mg/L of TCP.

This batch study was terminated after 45 days of continuous operation to examine changes to the beads. The beads appeared to be resilient, firm, and structurally sound.

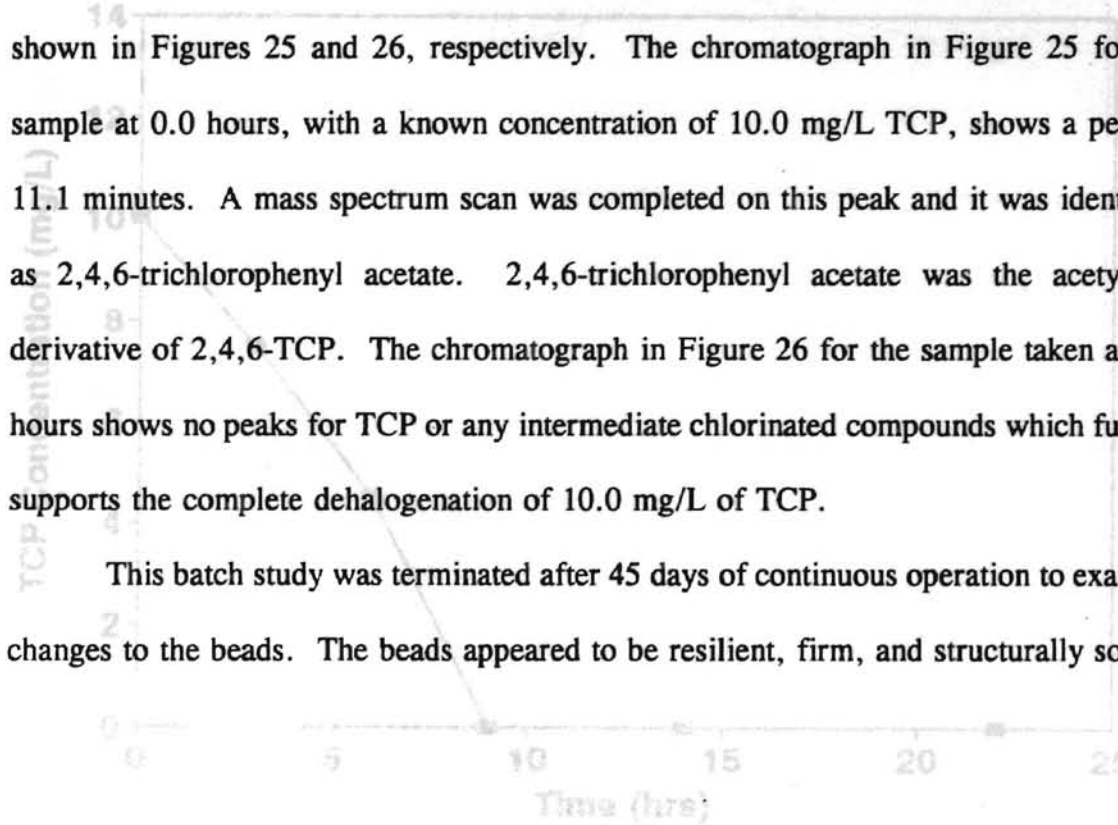


Figure 27 Biodegradation of TCP by Free Cells

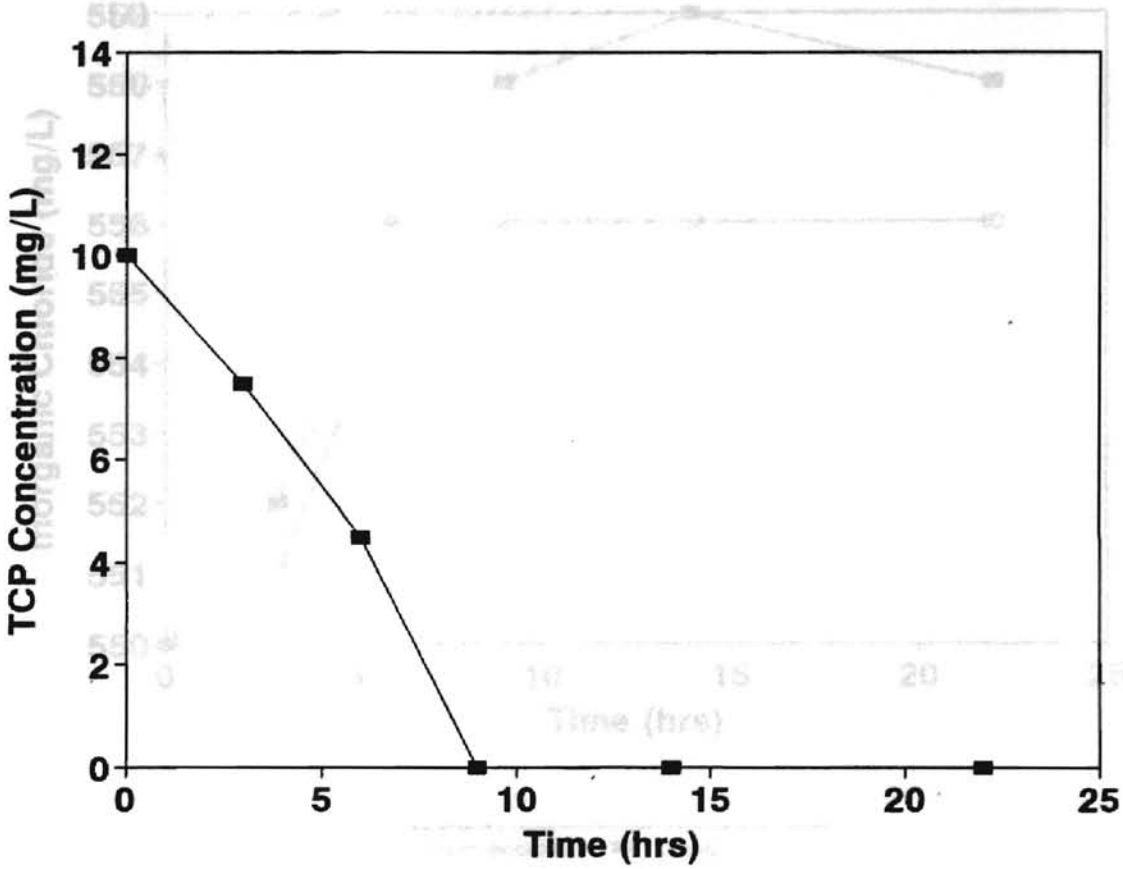


Figure 21. Biodegradation of TCP by Free Cells.

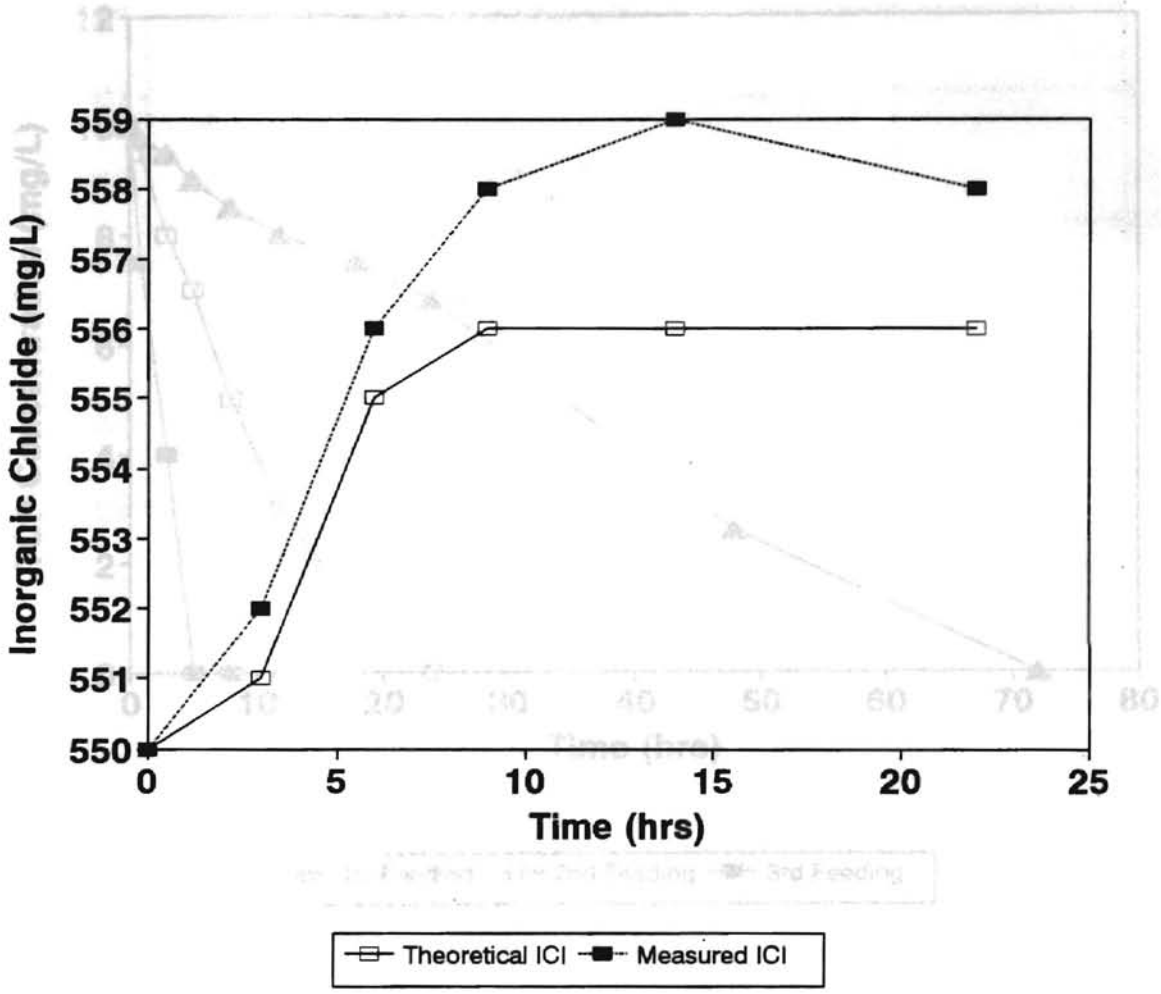


Figure 22. Inorganic Chloride Released by Free Cells.

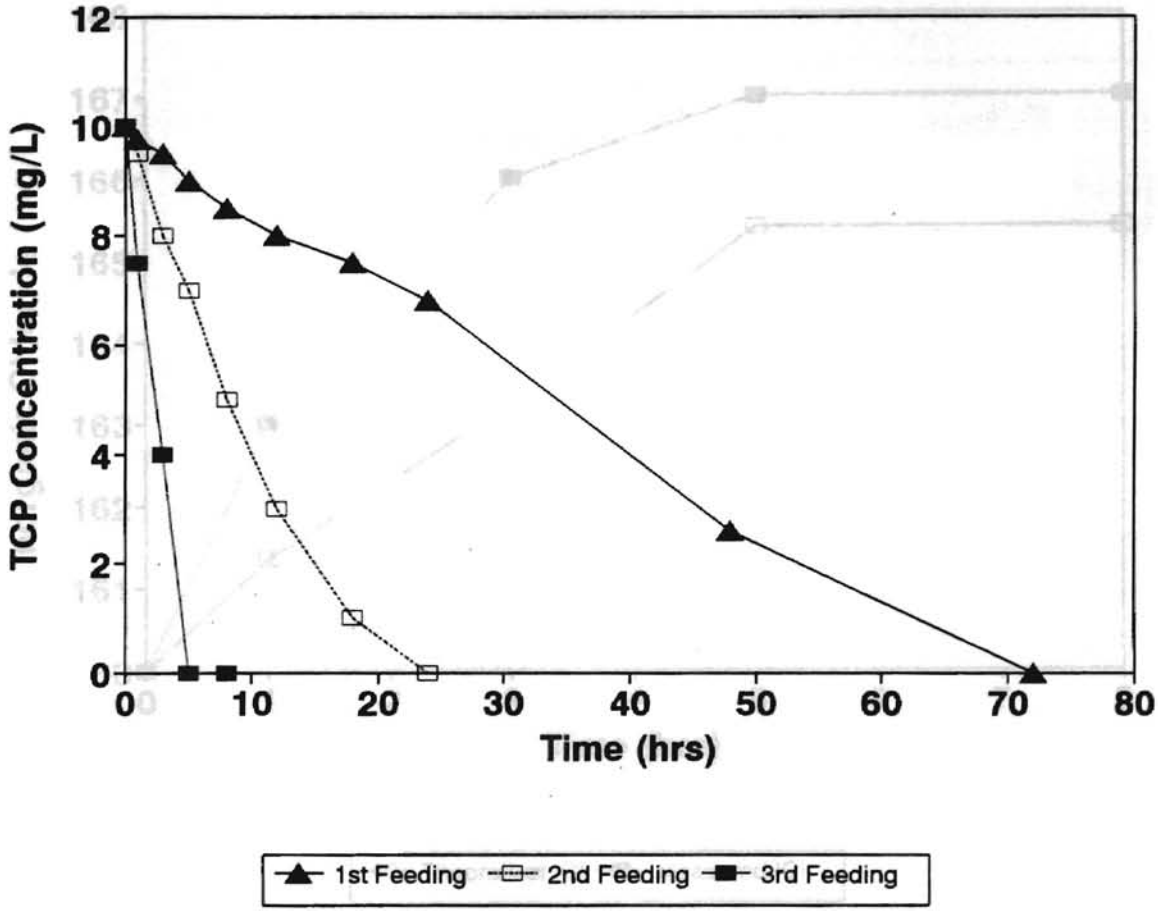


Figure 23. Biodegradation of TCP by Immobilized Cells.

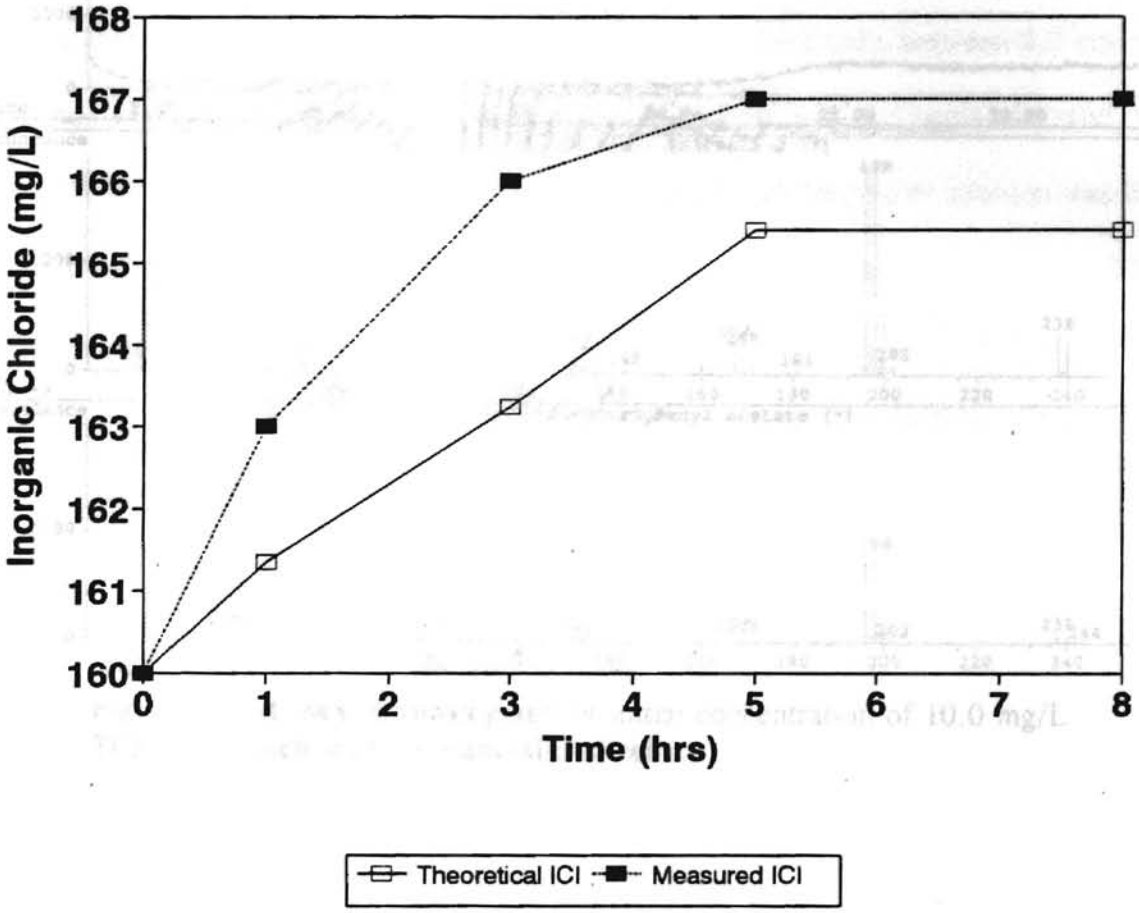


Figure 24. Inorganic Chloride Released by Immobilized Cells.

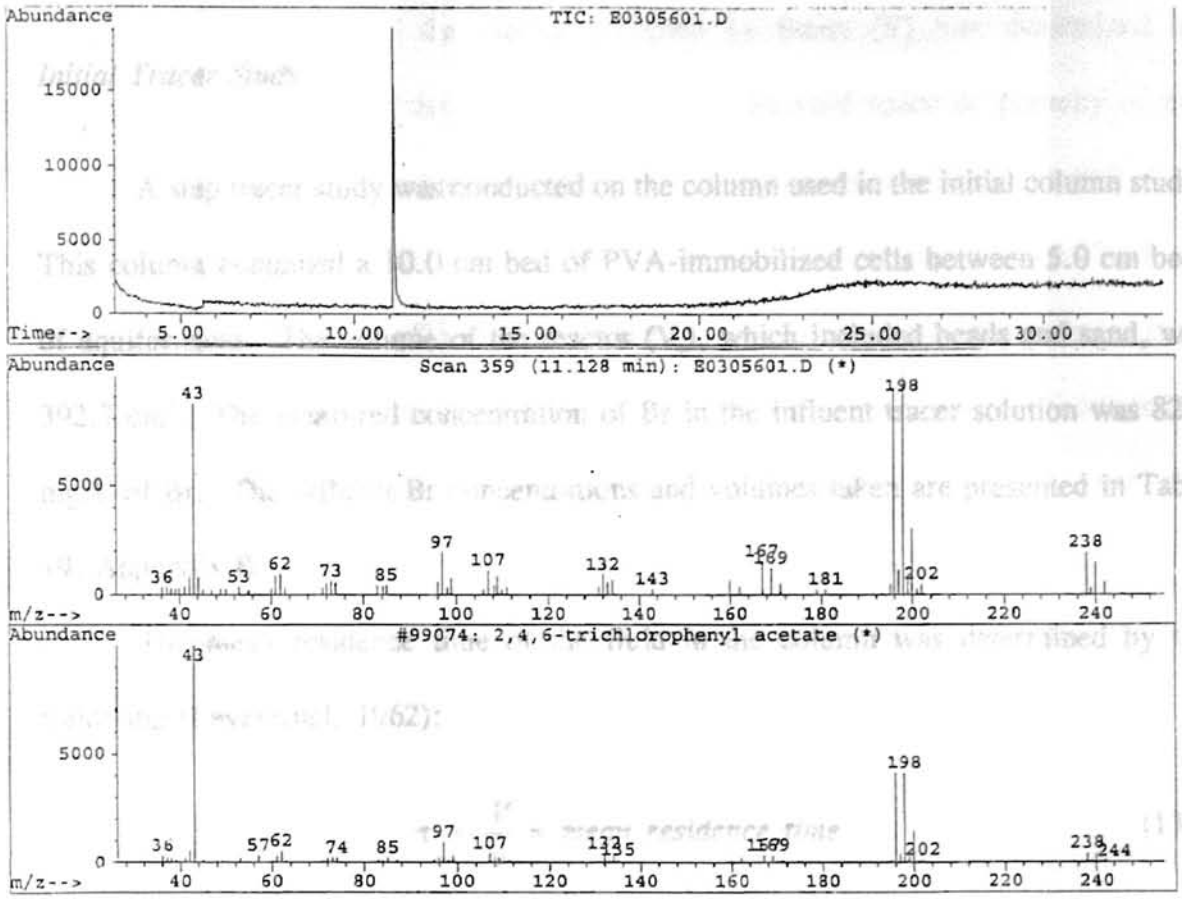


Figure 25. GC-MS chromatograph of initial concentration of 10.0 mg/L TCP from batch study of immobilized cells.

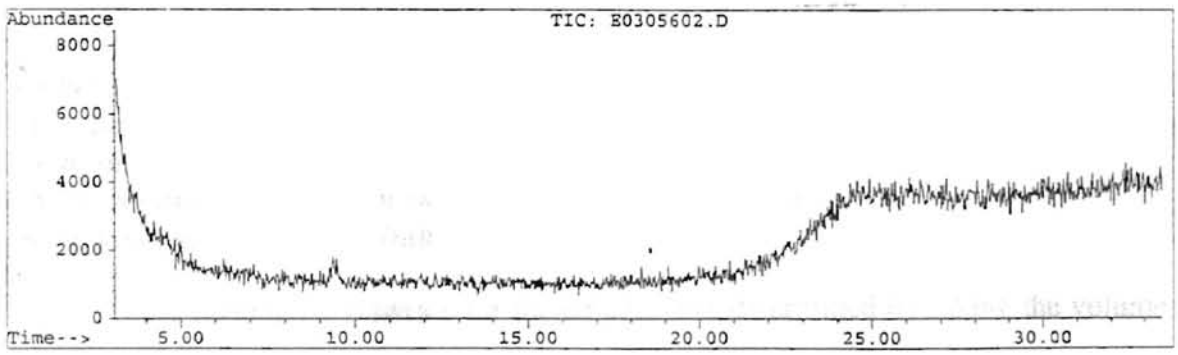


Figure 26. GC-MS chromatograph of sample from batch study of immobilized cells taken at 5.0 hrs.

Tracer Studies

Initial Tracer Study

A step tracer study was conducted on the column used in the initial column study. This column contained a 10.0 cm bed of PVA-immobilized cells between 5.0 cm beds of aquifer sand. The volume of the reactor (V_r), which included beads and sand, was 392.7 cm^3. The measured concentration of Br in the influent tracer solution was 82.0 mg/L of Br. The effluent Br concentrations and volumes taken are presented in Table 19, Appendix B.

The mean residence time of the fluid in the column was determined by the following (Levenspiel, 1962):

$$\tau = \frac{V}{\nu} = \textit{mean residence time} \qquad (13)$$

and the reduced time was determined by the following (Levenspiel, 1962):

$$\theta = \frac{t}{\tau} = \textit{reduced time} \qquad (14)$$

Where
t = time, min
τ = mean residence time, min
V = volume of the reactor occupied by fluids, mL or cm^3
ν = volumetric flow, mL/min

The volumetric flowrate for the column was determined by taking the volume of effluent collected during the tracer study and dividing by the number of minutes the study

lasted. The average volumetric flow for the column was determined to be 0.973 mL/min. The volume of the reactor occupied by fluids (V) was determined by multiplying the volume of the reactor (V_r) by the the void space or porosity of the column. The column was composed of 50% sand with a porosity of 30% and 50% PVA beads with a porosity of 25%. The overall void space within the column was 27.5% of the V_r. By using Equation (13) the mean residence time for the column was determined to be 111.1 minutes. This residence time (τ) was used to determine the reduced time (θ) for each data point from Equation (14). Tracer data are presented in Table 19, Appendix B.

The area under the curve of a plot of Effluent Bromide Concentrations vs. Effluent Volumes was the amount of Br recovered during the tracer study. The area under the curve in Figure 27 was determined by counting squares. The resulting area equalled 10.8 mg of Br. A mass balance of the Br concentration was determined by substracting from the total effluent volume of 349.75 mLs the volume of pore water displaced (108 mLs) and the volume of Br tracer solution remaining (108 mLs) in the column. The 133.75 mL influent volume of 82.0 mg/L Br solution resulted in a total input Br mass of 10.97 mg. This column had a 98% Br recovery.

The F curve ($F=C/C_0$ vs. θ) was plotted from the data in Table 19, Appendix B, and is shown in Figure 28. The F curve had three slopes which may have indicated the flow of Br through three different media zones within the column. Theoretically, this could be explained as follows: the first slope represents the flow through the first bed of sand; the flat area of the curve represents the flow through the layer of beads; and, the

was the flow through the upper bed of sand. The inflection points of the curve represent the interfaces between each bed. The first and third slopes were similar, indicating the same amount of dispersion. The second slope, which was almost flat, indicated a very large amount of dispersion. The vessel dispersion number $D/\mu L$ was approximated to be 0.2 by comparing the slopes of the curve from Figure 28 to those predicted by Levenspiel (1962). The lower incline of the slopes indicated a large amount of dispersion within the column. The first segment of the curve reached its point of maximum slope at $\theta = 1.0$; the second segment of the curve reached its point of maximum slope at $\theta = 1.75$; and, the third segment of the curve reached its point of maximum slope at $\theta = 2.5$.

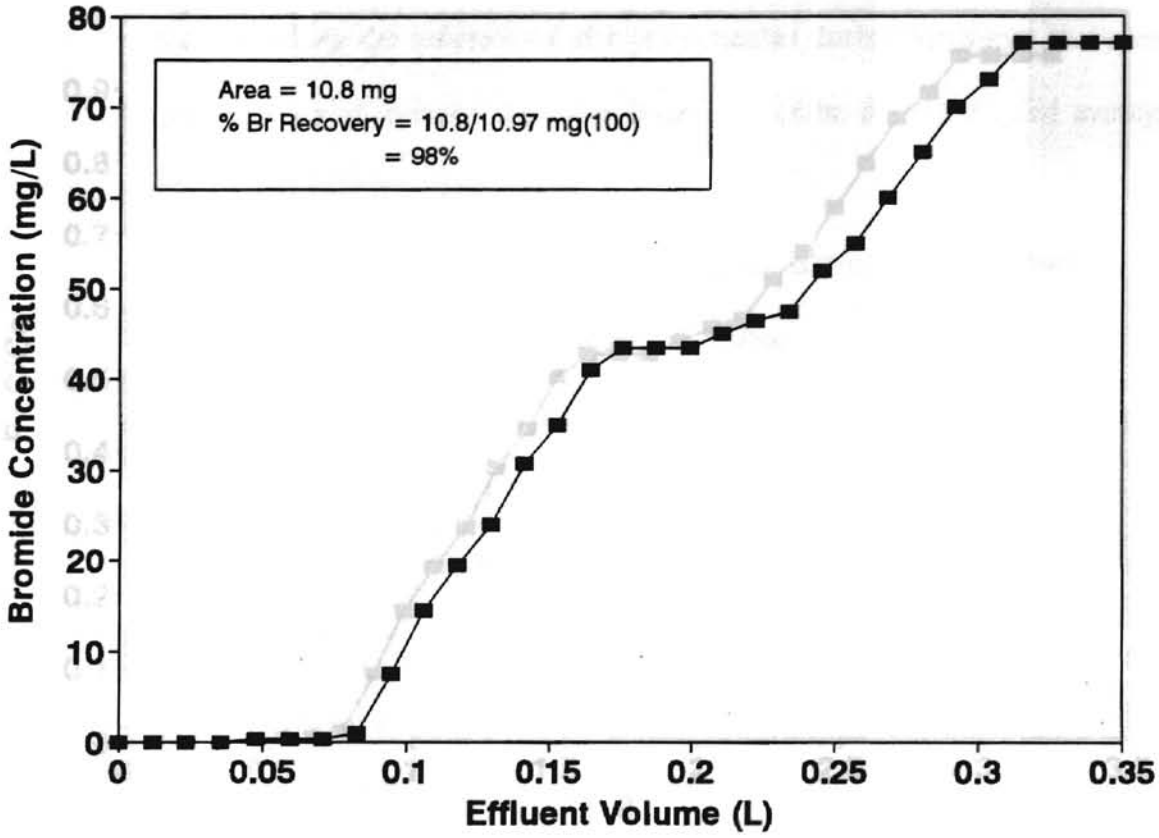


Figure 27. Tracer study for initial column study -- % Bromide recovery.

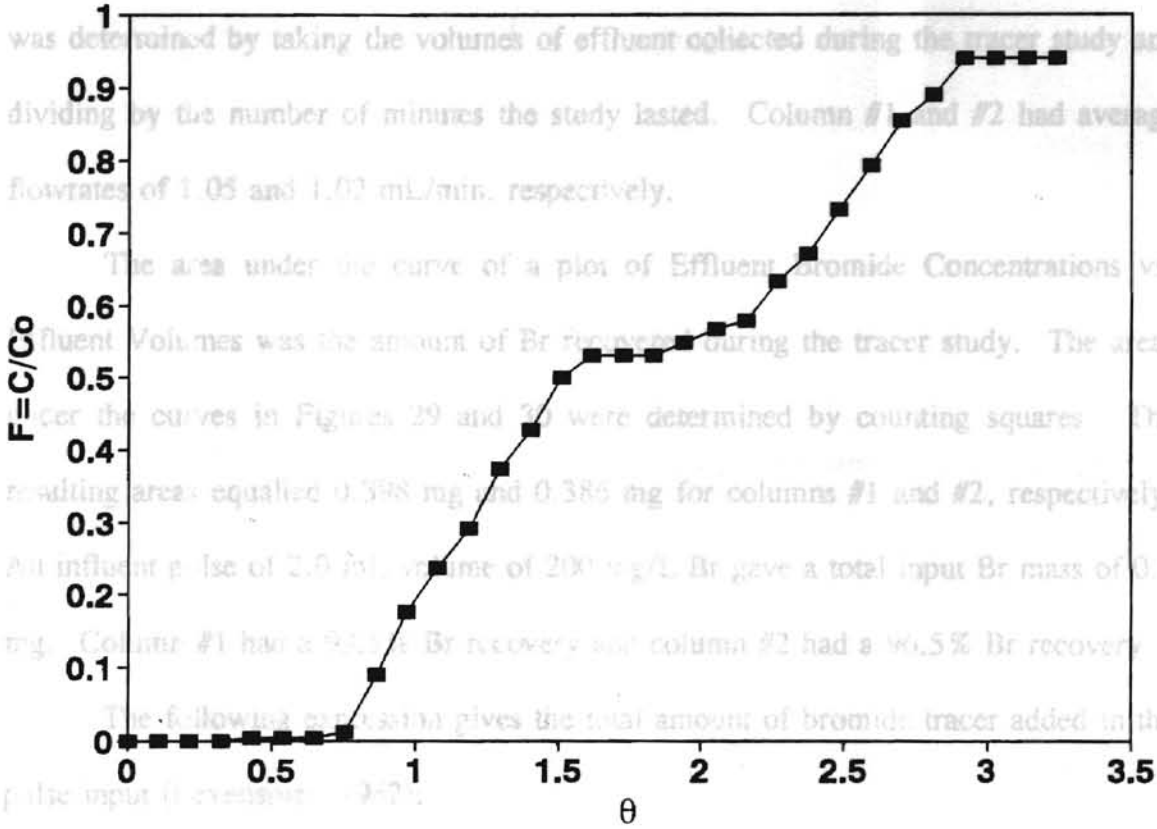


Figure 28. Tracer study for initial column study -- F curve.

Final Tracer Study

The bromide concentrations analyzed from the pulse tracer study for columns #1 and #2 are presented in Tables 20 and 21, Appendix B. The final column study used two columns containing PVA-immobilized cells only. Column #1 had a volume of 157 cm^3 and column #2 had a volume of 392.7 cm^3. The volumetric flowrate for each column was determined by taking the volumes of effluent collected during the tracer study and dividing by the number of minutes the study lasted. Column #1 and #2 had average flowrates of 1.05 and 1.02 mL/min, respectively.

The area under the curve of a plot of Effluent Bromide Concentrations vs. Effluent Volumes was the amount of Br recovered during the tracer study. The areas under the curves in Figures 29 and 30 were determined by counting squares. The resulting areas equalled 0.398 mg and 0.386 mg for columns #1 and #2, respectively. An influent pulse of 2.0 mL volume of 200 mg/L Br gave a total input Br mass of 0.4 mg. Column #1 had a 99.5% Br recovery and column #2 had a 96.5% Br recovery.

The following expression gives the total amount of bromide tracer added in the pulse input (Levenspiel, 1962):

$$\sum C \, \Delta t \tag{15}$$

Where
C = bromide concentration (mg/L)
t = time

The mean residence time was determined from the expression (Levenspiel, 1962):

$$\tau = \frac{\sum tC}{\sum C} = \textit{mean residence time} \tag{16}$$

Where
τ = mean residence time (min)

and

$$\theta = \frac{t}{\tau} = \textit{reduced time} \tag{17}$$

and

$$E = \frac{\tau C}{\sum C\,\Delta t} \tag{18}$$

E, $\Sigma C\,\Delta t$, τ, and θ were calculated and the values are listed in Tables 20 and 21, Appendix B. A plot of E vs θ for columns #1 and #2 are in Figures 31 and 32, respectively. The area under each of the curves in Figures 31 and 32 were determined by counting squares to be equal to 1.0.

The vessel dispersion number $D/\mu L$ was calculated from the following expression (Levenspiel, 1962):

$$\sigma^2 = \frac{\sum \theta^2 E}{\sum E} - 1 \tag{19}$$

and

$$\sigma^2 = 2\frac{D}{\mu L} - 2\left(\frac{D}{\mu L}\right)^2 \left(1 - e^{\frac{-\mu L}{D}}\right) \tag{20}$$

The second term on the right of Equation 20 was ignored because its value was very small and an approximation was made:

$$\frac{D}{\mu L} \approx \frac{\sigma^2}{2} \tag{21}$$

Correcting for the term ignored, the value of $D/\mu L$ was found by trial and error. Column #1 had a large amount of dispersion, as shown in Figure 31 and from the $D/\mu L$ value of 0.1933 (Levenspiel, 1962). Column #1 had rapidly risen to a peak where θ was 1.0, but then the peak broadened on its down slope with the curve tailing to the right. Column #1 was 5.0 cm in diameter and 8.0 cm in height. The shortness of the column in relationship to its diameter gave it some characteristics of a mixed reactor. This was evidenced by the broad base of the curve indicating that mixing occurred within the column. Column #2, had an intermediate amount of dispersion as shown in Figure 32 and from the $D/\mu L$ value of 0.0632 (Levenspiel, 1962). A walling effect potentially occurred in column #2 as evidenced in Figure 32 by the very low slope fluctuating about the 0.5 E value. The curve then rapidly rose to a steep peak that was symmetrical about the point where θ equals 1.1. The steep symmetrical curve was similar to that of a plug-flow reactor.

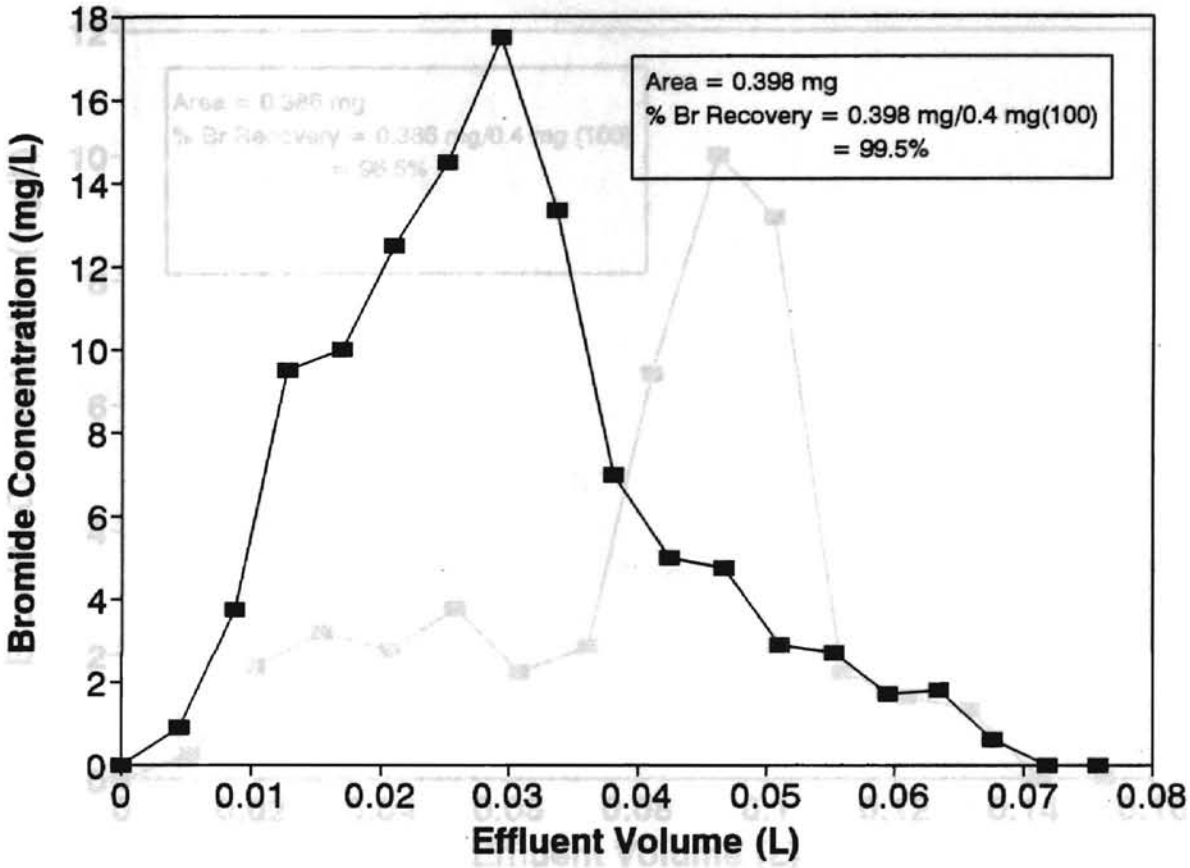


Figure 29. Tracer study for final column study--% Bromide recovery for column #1.

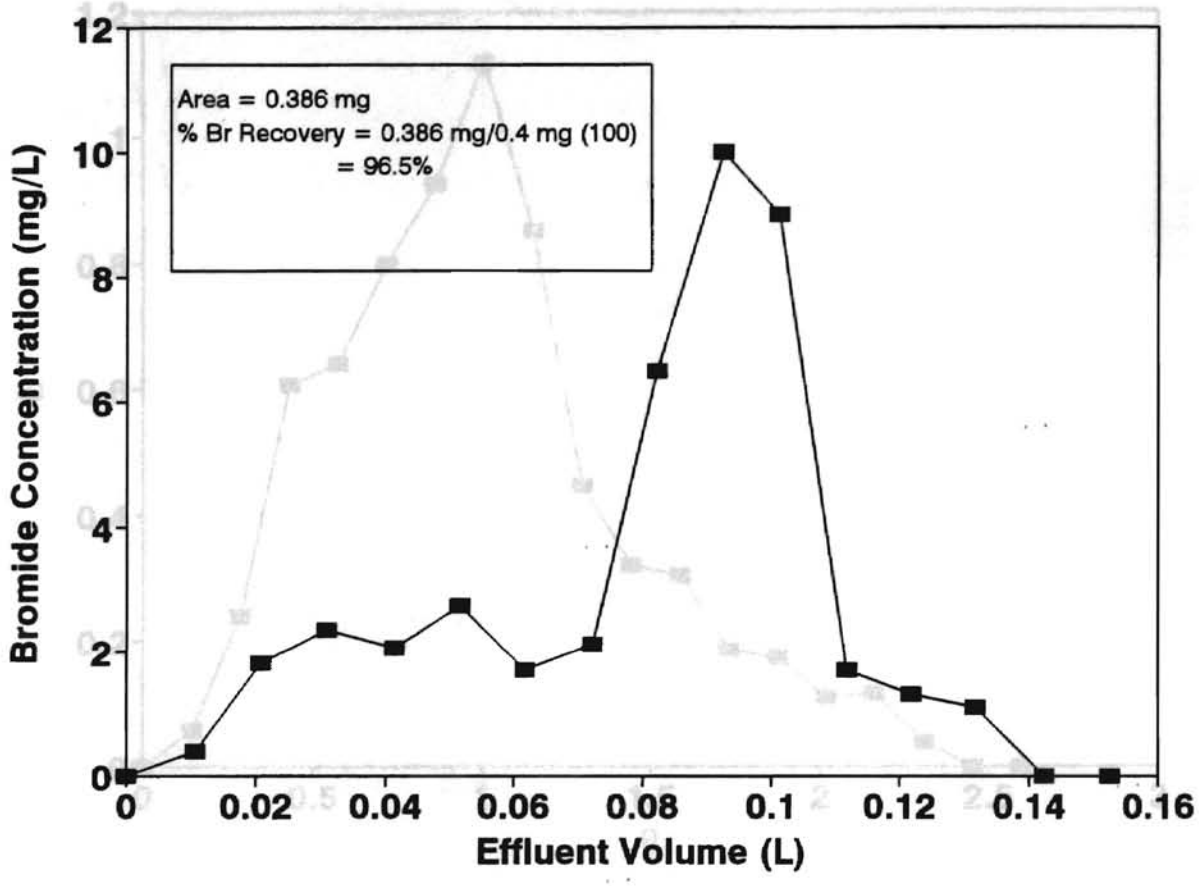


Figure 30. Tracer study for final column study--% Bromide recovery for column #2.

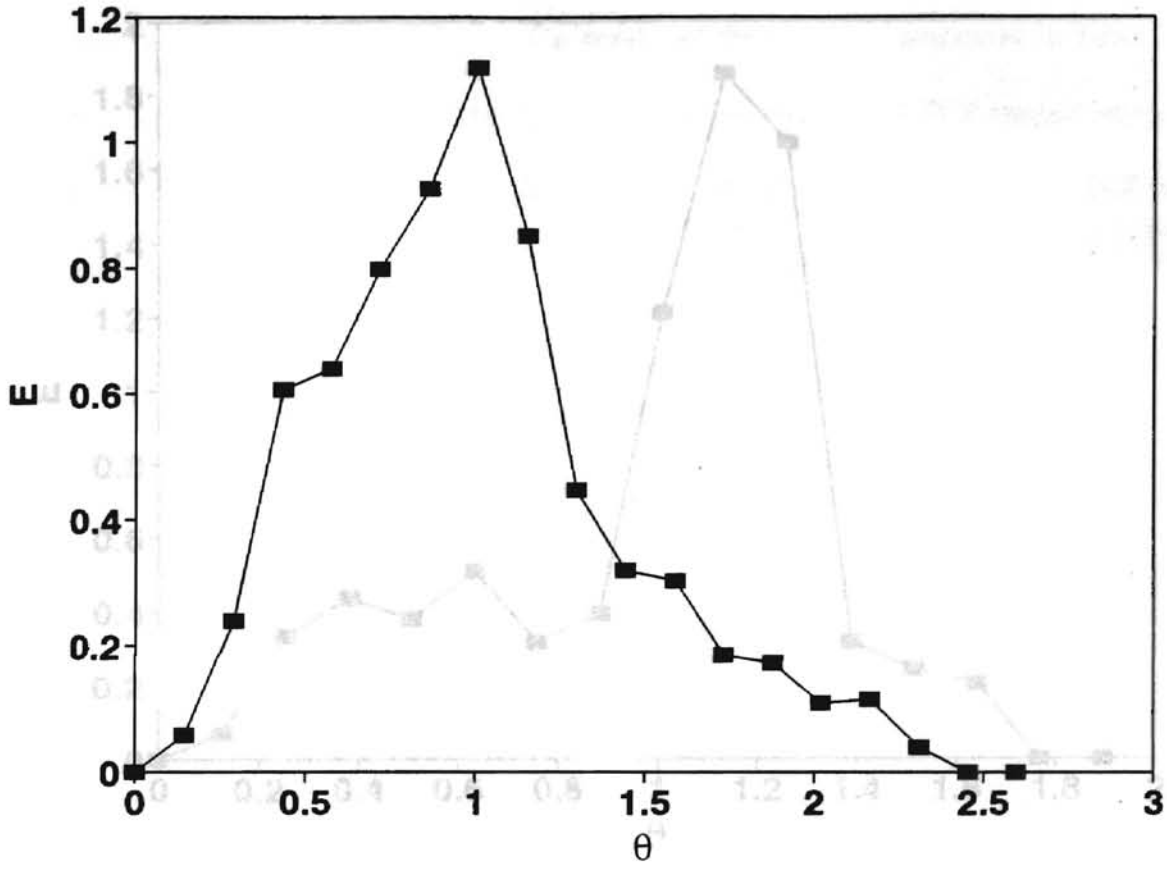


Figure 31. Tracer study for final column study, column #1 -- E curve.

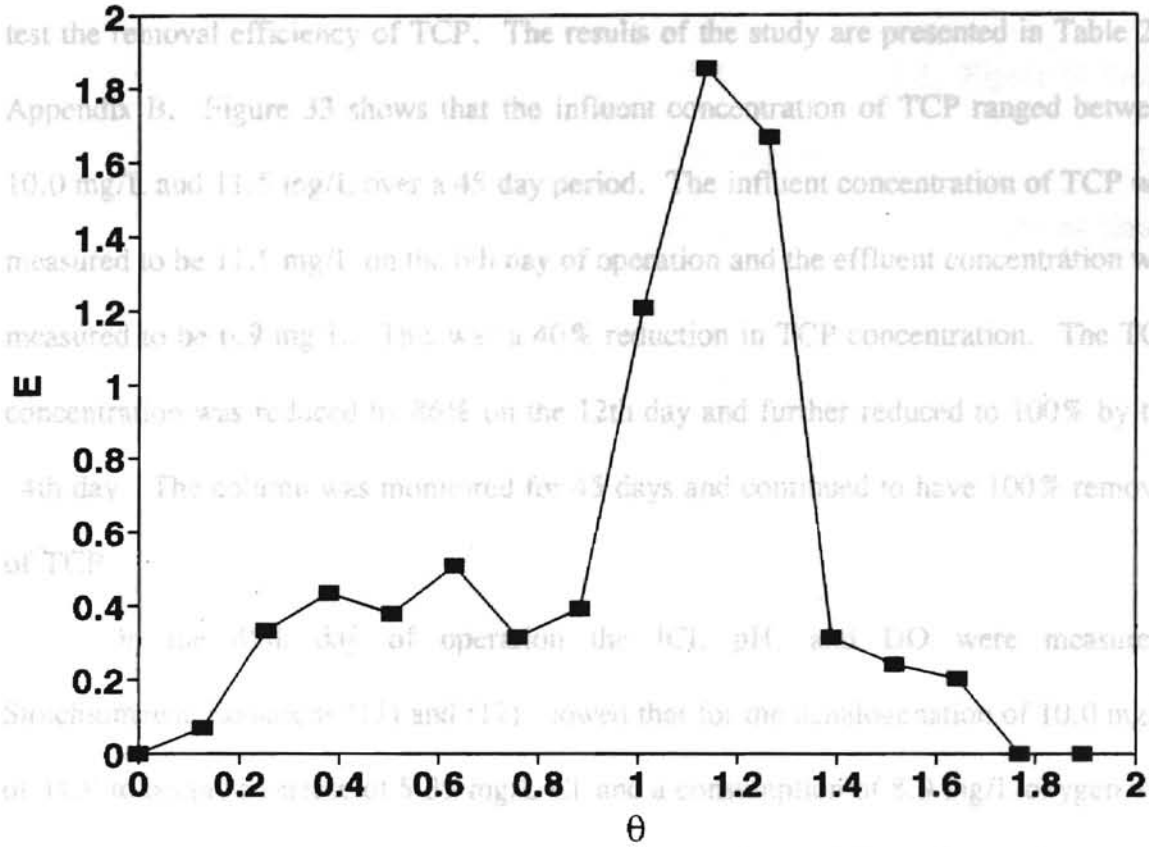


Figure 32. Tracer study for final column study, column #2 -- E curve.

Column Studies

Initial Column Study

The column in the initial column study contained a 10.0 cm bed of PVA-immobilized cells between two 5.0 cm beds of aquifer sand. This column was used to test the removal efficiency of TCP. The results of the study are presented in Table 22, Appendix B. Figure 33 shows that the influent concentration of TCP ranged between 10.0 mg/L and 11.5 mg/L over a 45 day period. The influent concentration of TCP was measured to be 11.5 mg/L on the 6th day of operation and the effluent concentration was measured to be 6.9 mg/L. This was a 40% reduction in TCP concentration. The TCP concentration was reduced by 86% on the 12th day and further reduced to 100% by the 14th day. The column was monitored for 45 days and continued to have 100% removal of TCP.

On the 45th day of operation the ICl, pH, and DO were measured. Stoichiometric Equations (11) and (12) showed that for the dehalogenation of 10.0 mg/L of TCP to occur, a release of 5.39 mg/L Cl^- and a consumption of 8.9 mg/L oxygen was required. The ICl influent concentration was measured to be 160 mg/L and the effluent concentration was measured to be 167 mg/L. This was an ICl increase of 7 mg/L (160-167). The ICl increase supports the dehalogenation of TCP.

A pH curve was prepared by titrating a 1.0 L sample of 10.0 mg/L TCP influent feed solution with 0.1 N Hydrochloric Acid (HCl) solution (Table 23, Appendix B). The 0.1 N HCl solution was prepared by adding 8.3 mLs of concentrated HCl to distilled water to make a 1.0 liter solution (Standard Methods, 1975). The pH measurements of

the feed solution were plotted against the milliliters of 0.1 N HCl used in the titration (Figure 34). Each mL of titrant contained 3.55 mg Cl^- as follows:

$$0.1\ N\ HCl = 0.1\ M\ HCl = 0.1\ \frac{mole}{L}\left(\frac{35.5\ g\ Cl^-}{mole\ HCl}\right) = 3.55\ \frac{mg\ Cl^-}{mL\ titrant} \qquad (22)$$

The influent had a pH of 8.3 and the effluent had a pH of 7.8. Figure 34 shows that a 2.0 mL volume of titrant (0.1 N HCl) was required to obtain this same pH decrease for a 1.0 L sample of influent feed solution. The 2.0 mL volume of titrant needed per 1.0 L sample had a concentration of 7.1 mg/L of Cl^- (2.0 ml/L X 3.55 mg/ml). The 7.1 mg/L Cl^- needed to decrease the pH matched the 7.0 mg/L Cl- concentration liberated by the dehalogenation of TCP. This further supports the complete halogenation of TCP.

The influent had a DO of 8.6 mg/L and the effluent had a DO of 2.5 mg/L. The decrease in DO indicated aerobic activity. The 300 mL samples for DO measurement took 5.0 hours to collect. The samples were exposed to the air during the 5.0 hours which gave higher measured oxygen concentrations than were determined stoichiometrically. This was the only available method to measure DO.

Influent and effluent samples were collected on the 45th day of operation and extracted using the Voss (1981) method as previously described. The Voss extraction method produced acetylated derivatives of the original chlorinated compounds. The samples were analyzed by GC-ECD which showed an influent TCP concentration of 10.0 mg/L and an effluent TCP concentration of zero. The same samples were analyzed by GC-MS and resulting chromatographs for the influent and effluent are shown in Figures

35 and 36, respectively. The chromatograph in Figure 35 for the influent sample, with a measured concentration of 10.0 mg/L TCP, shows a peak at 11.1 minutes which was identified as 2,4,6-trichlorophenyl acetate. 2,4,6-trichlorophenyl acetate was the acetylated derivative of 2,4,6-TCP. The chromatograph in Figure 36 for the effluent sample shows no peaks for TCP or any intermediate compounds which supports dehalogenation of the 10.0 mg/L TCP.

This column study was terminated after 45 days of continuous operation to examine changes to the beads. The beads appeared to be resilient, firm, and structurally sound.

Final Column Study

The final column study was conducted for the purpose of comparing removal efficiencies of TCP between two columns that varied in size. A DAPI stain was first conducted on thin sections of PVA-immobilized cells. The bacteria fluoresced under the epifluorescence microscope. This verified that organisms containing DNA were immobilized within the beads prior to setting up columns #1 and #2. Column #1 had a bed height of 8 cm, a diameter of 5 cm, a volume of 157 cm^3, a porosity of 25%, and a theoretical hydraulic retention time (HRT) of 39 minutes. Column #2 had a bed height of 20 cm, a diameter of 5 cm, a volume of 392.7 cm^3, a porosity of 25%, and a theoretical hydraulic retention time (HRT) of 98 minutes. The results of the studies are presented in Table 24, Appendix B. Figure 37 shows the influent concentration and effluent concentrations over a period of 14 days. The influent TCP concentration ranged

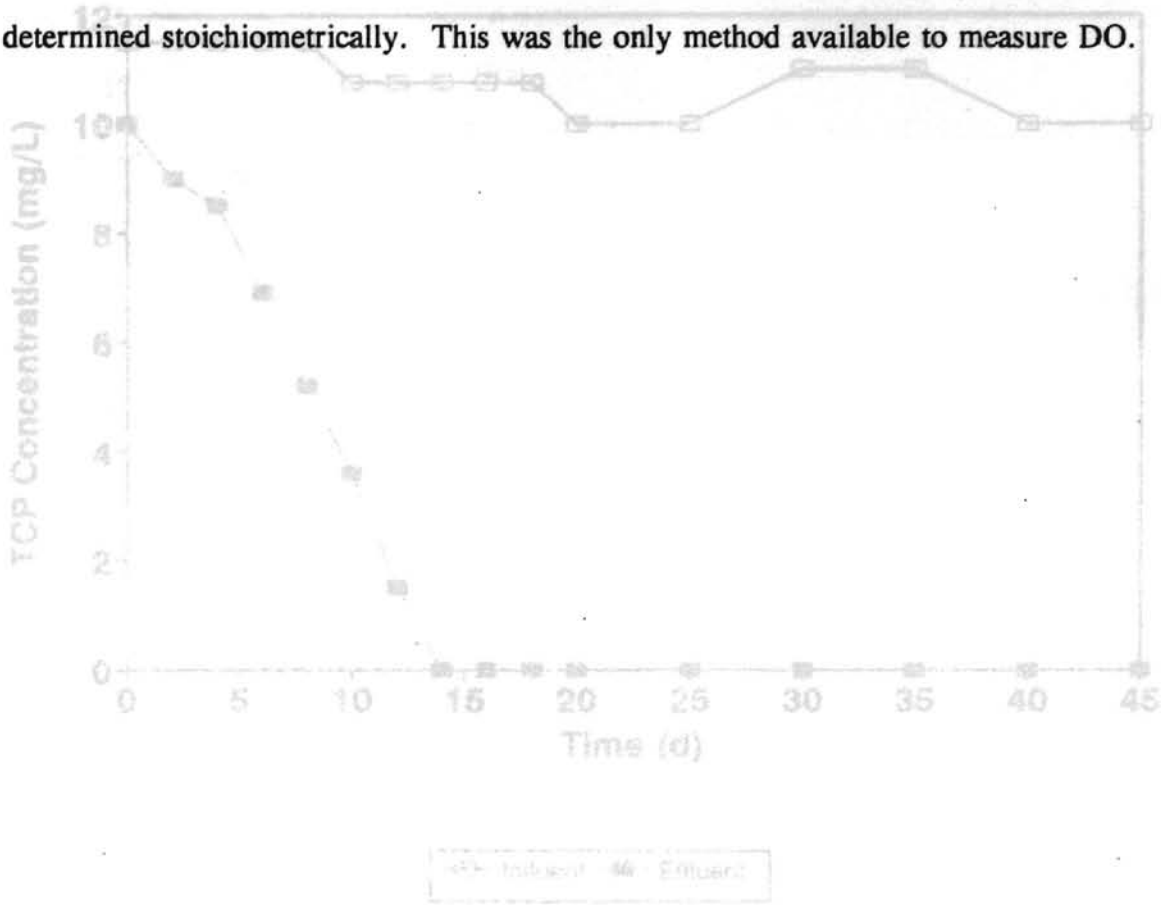
from 10.0 mg/L to 11.0 mg/L. It took a period of time (8-10 days) for the columns to reach zero concentrations in the effluent. Column #1 had 100% removal within 10 days and column #2 had 100% removal within 8 days.

The ICl concentrations were measured in the influent and effluent of column #1 and #2, and are shown in Figure 38. The 14th day of operation shows the ICl influent concentration was measured to be 158 mg/L and the effluent concentrations were measured to be 164 mg/L for both column #1 and #2. This was an ICl increase of 6 mg/L (158-164). The measured increases were very close in value to theoretical ICl values predicted from TCP concentrations. Figures 39 and 40 compare the theoretical and measured ICl increases from columns #1 and #2 to the % TCP removal. The curves are both very close in value, which tend to confirm that dehalogenation of TCP was occurring.

The influent feed solution had pH ranges from 8.1 to 8.3. Column #1 and #2 had effluent pH values from 7.5 to 7.8. A comparison of pH values are shown in Figure 41. From the pH curve, Figure 34, the drop in pH from 8.3 in the influent to 7.5 pH in the effluents shows that a 3.0 mL volume of 0.1 N HCl would be required. This is a 10.65 mg/L Cl^- concentration (3.0 mL/L X 3.55 mg/mL) which was similar in value to the ICl concentration of 6.0 mg/L released from the dehalogenation of 10.0 mg/L TCP. This tends to support the dehalogenation of TCP.

The influent had DO measurements ranging from 8.4 mg/L to 8.6 mg/L for both columns. The effluents had DO measurements decreasing to 6.6 mg/L and 7.5 mg/L, for columns #1 and #2 respectively, and are shown in Figure 42. The decrease in DO

indicated aerobic activity. Both curves steadily decreased over time. The 300 mL samples for DO measurement took 5.0 hours to collect. The samples were exposed to the air during the 5.0 hours, which gave higher measured oxygen concentrations than determined stoichiometrically. This was the only method available to measure DO.



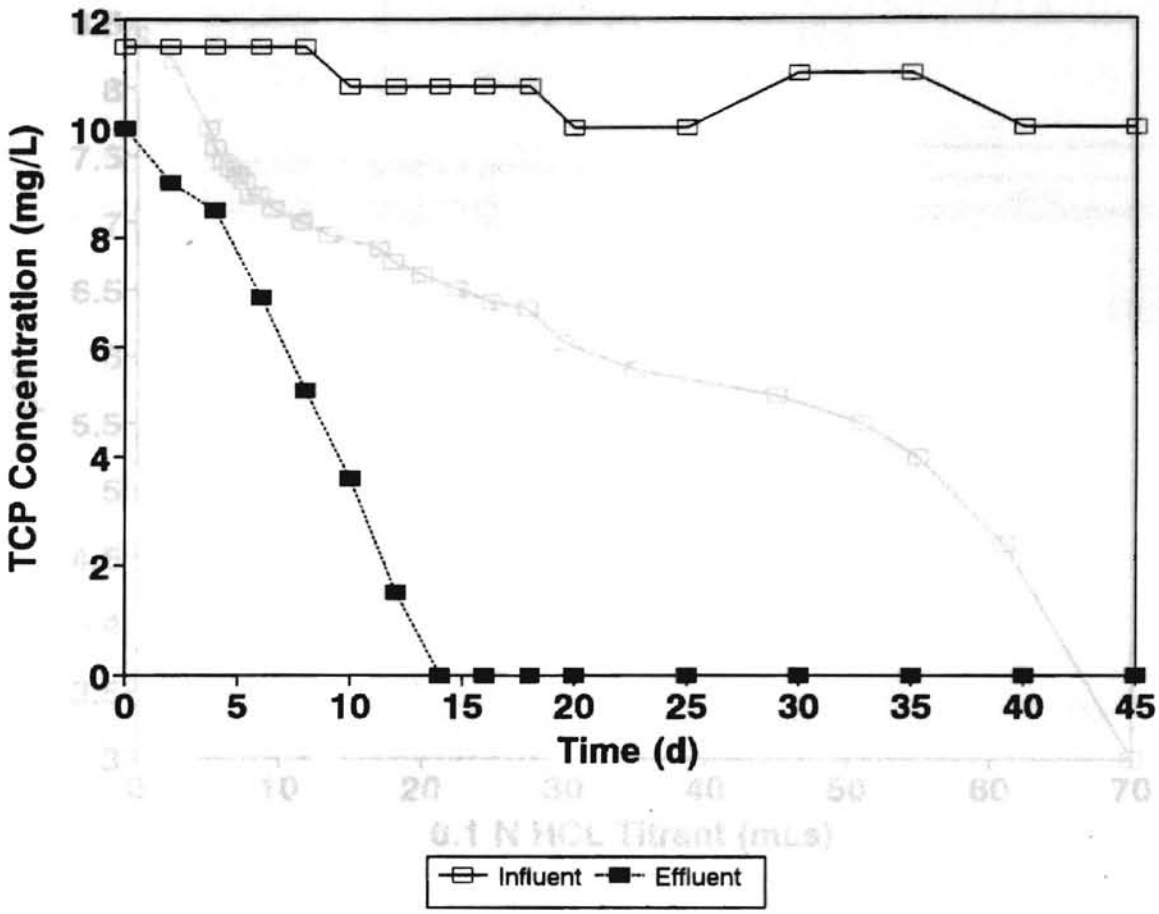


Figure 33. Initial column study -- Biodegradation of TCP.

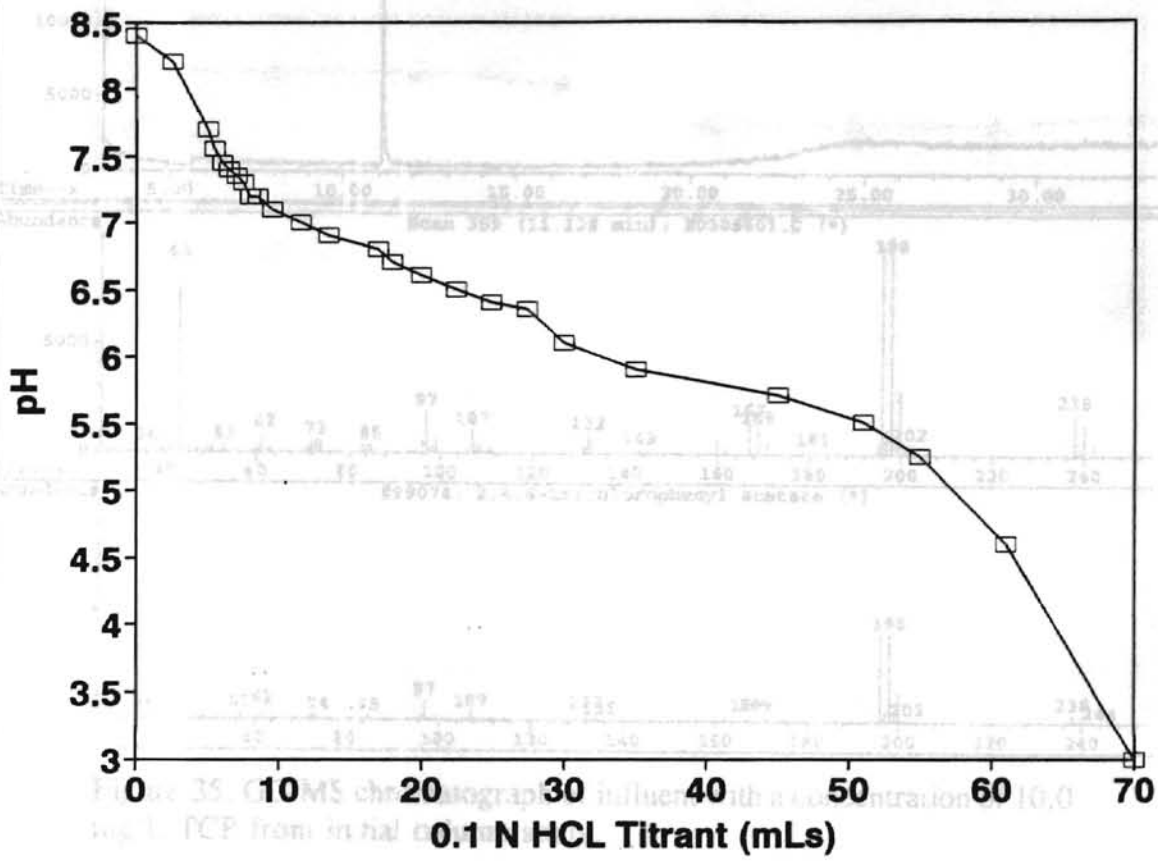


Figure 34. pH curve.

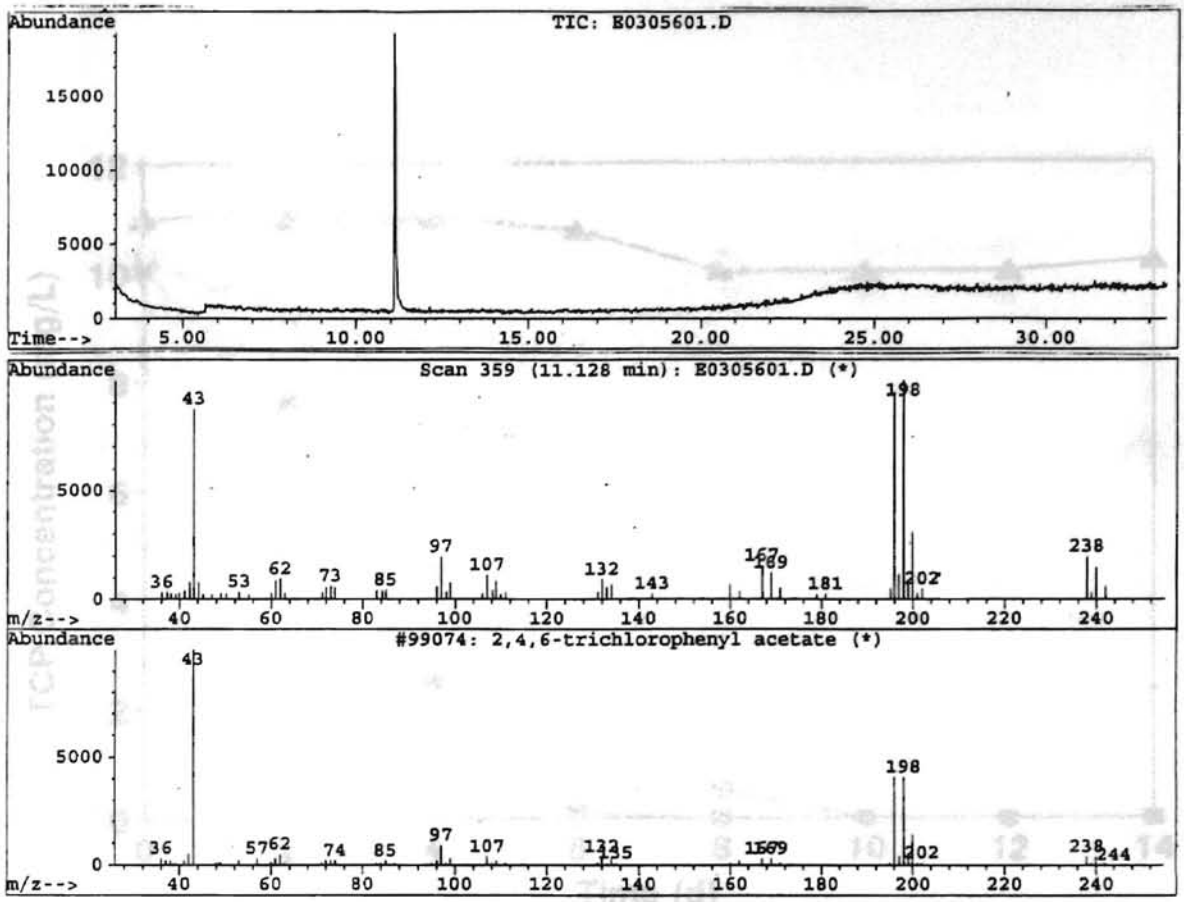


Figure 35. GC-MS chromatograph of influent with a concentration of 10.0 mg/L TCP from initial column study.

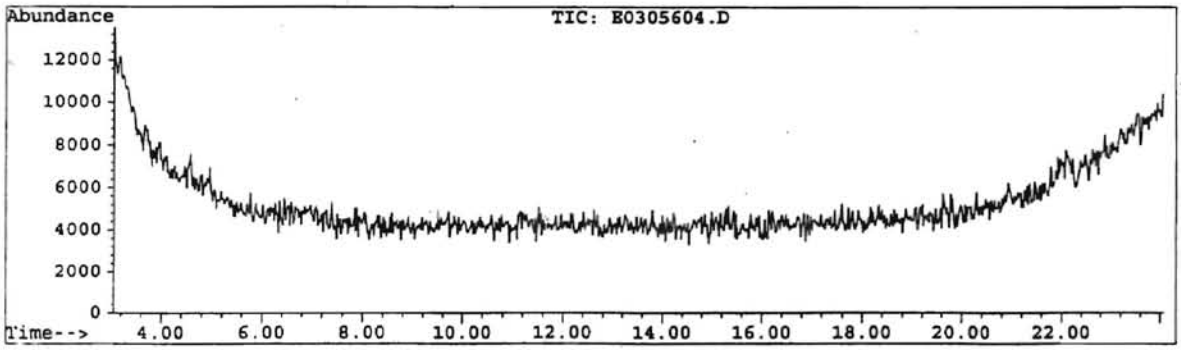


Figure 36. GC-MS chromatograph of effluent sample taken from the initial column study.

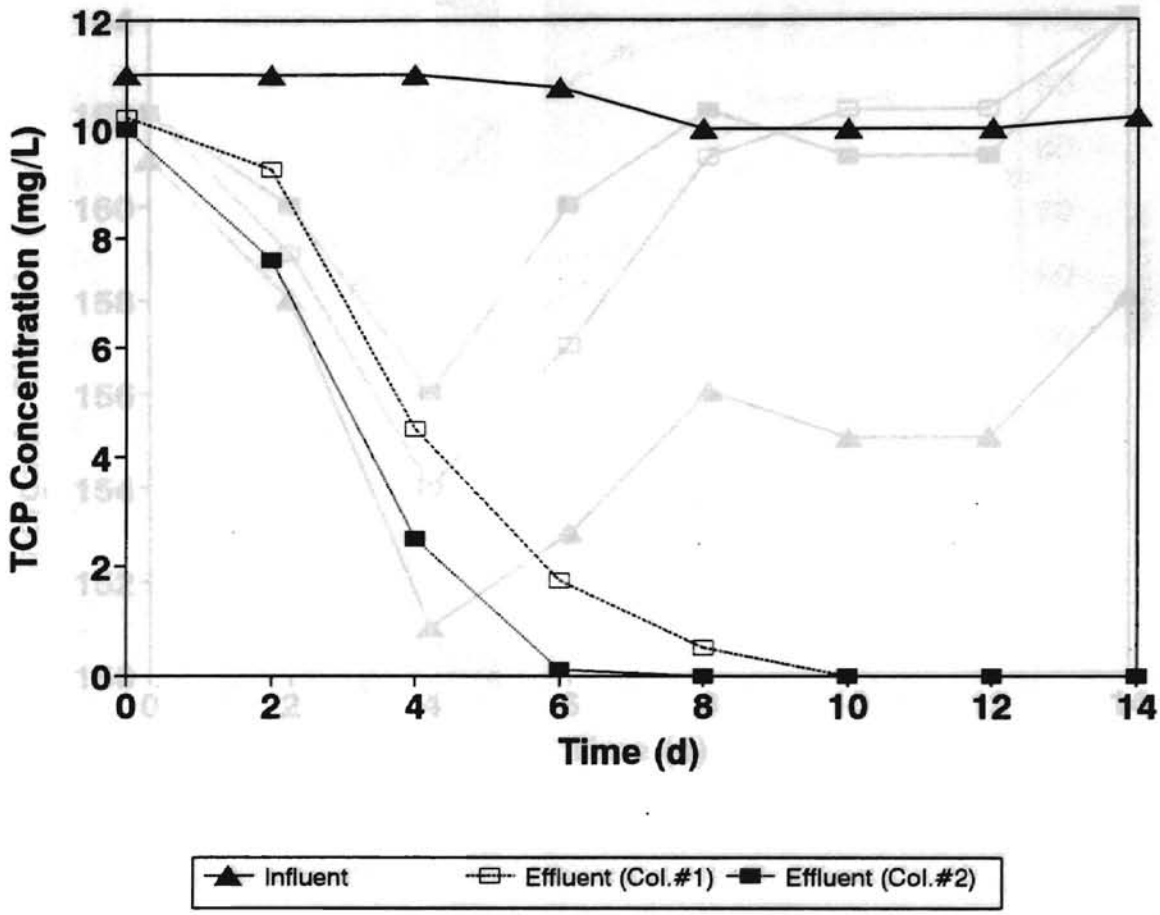


Figure 37. Final column study -- Biodegradation of TCP from columns #1 and #2.

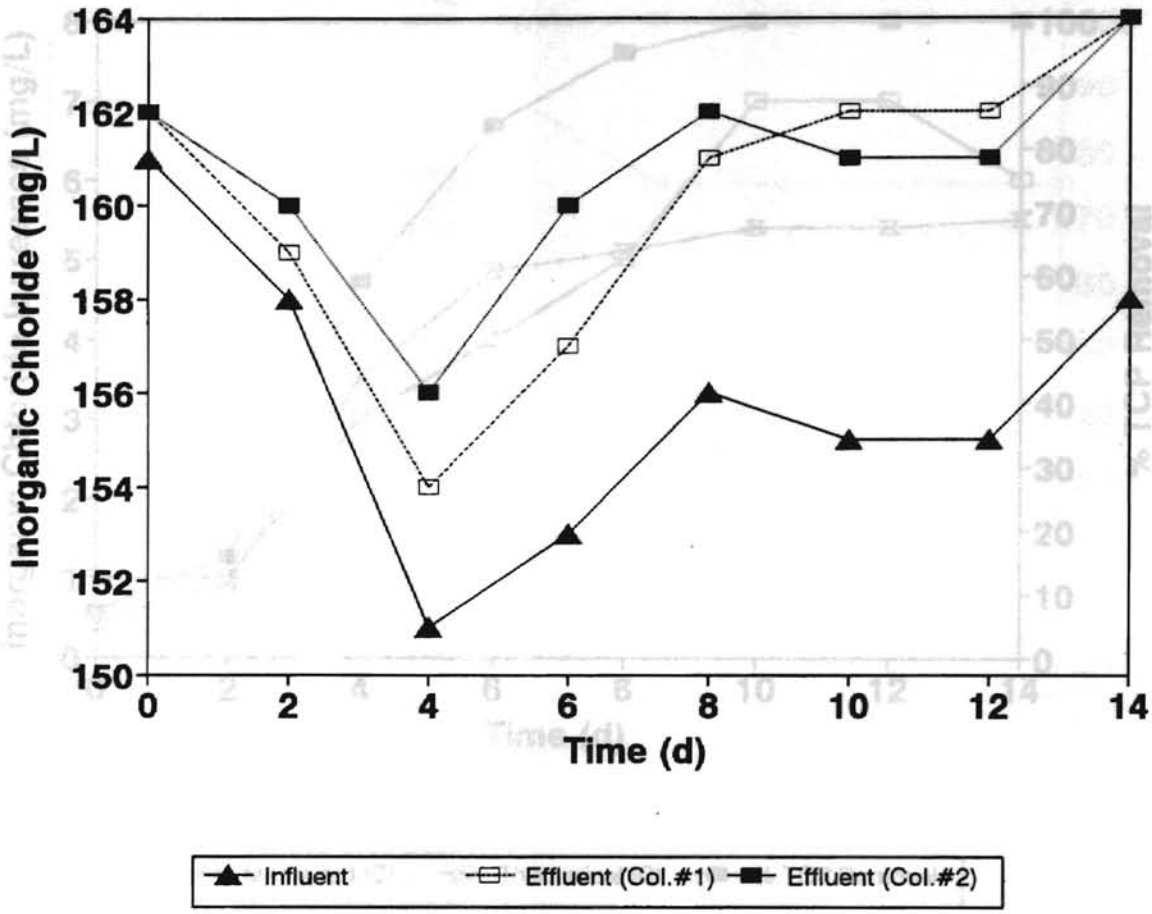


Figure 38. Final column study -- Chloride releases from columns #1 and #2.

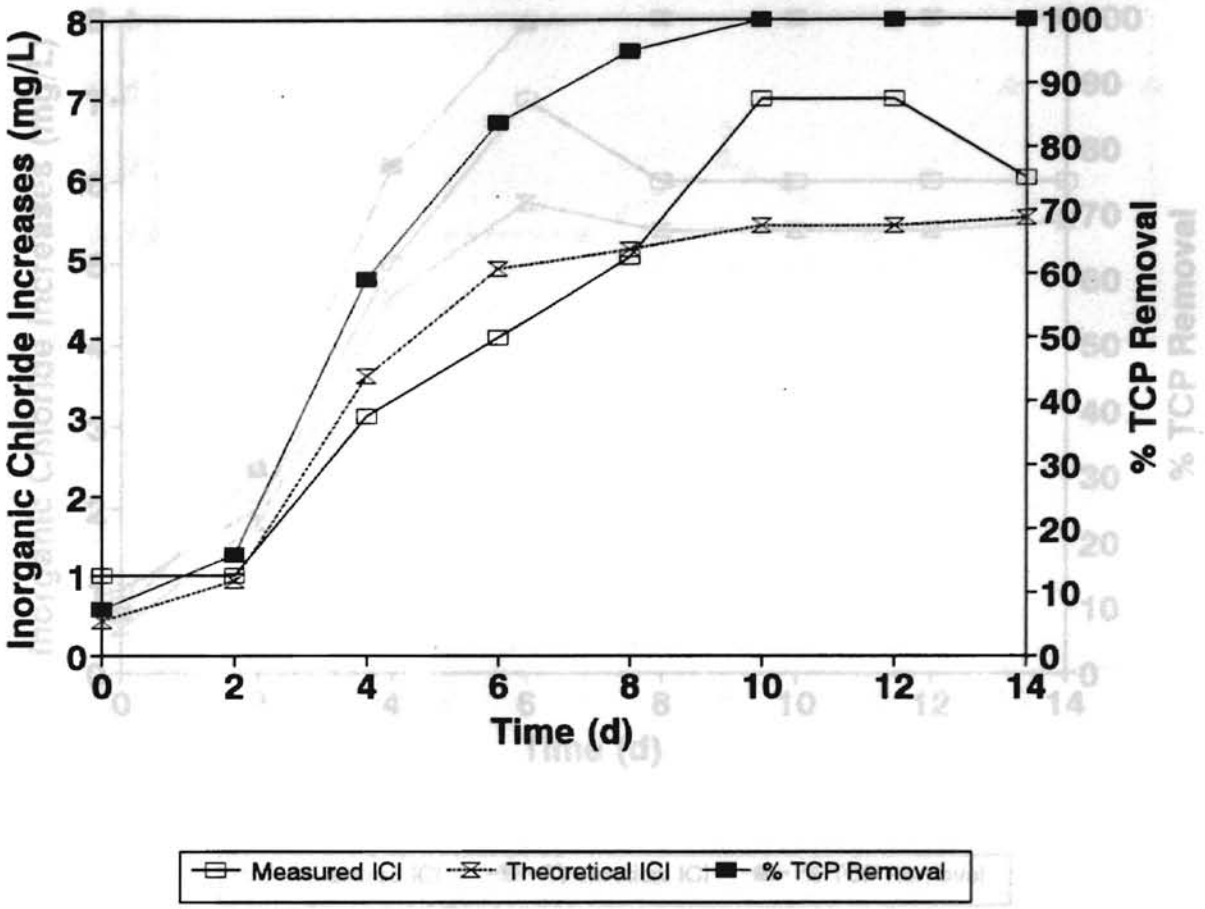


Figure 39. Final column study, column #1 -- Comparison of %TCP removal, measured and theoretical inorganic chloride releases.

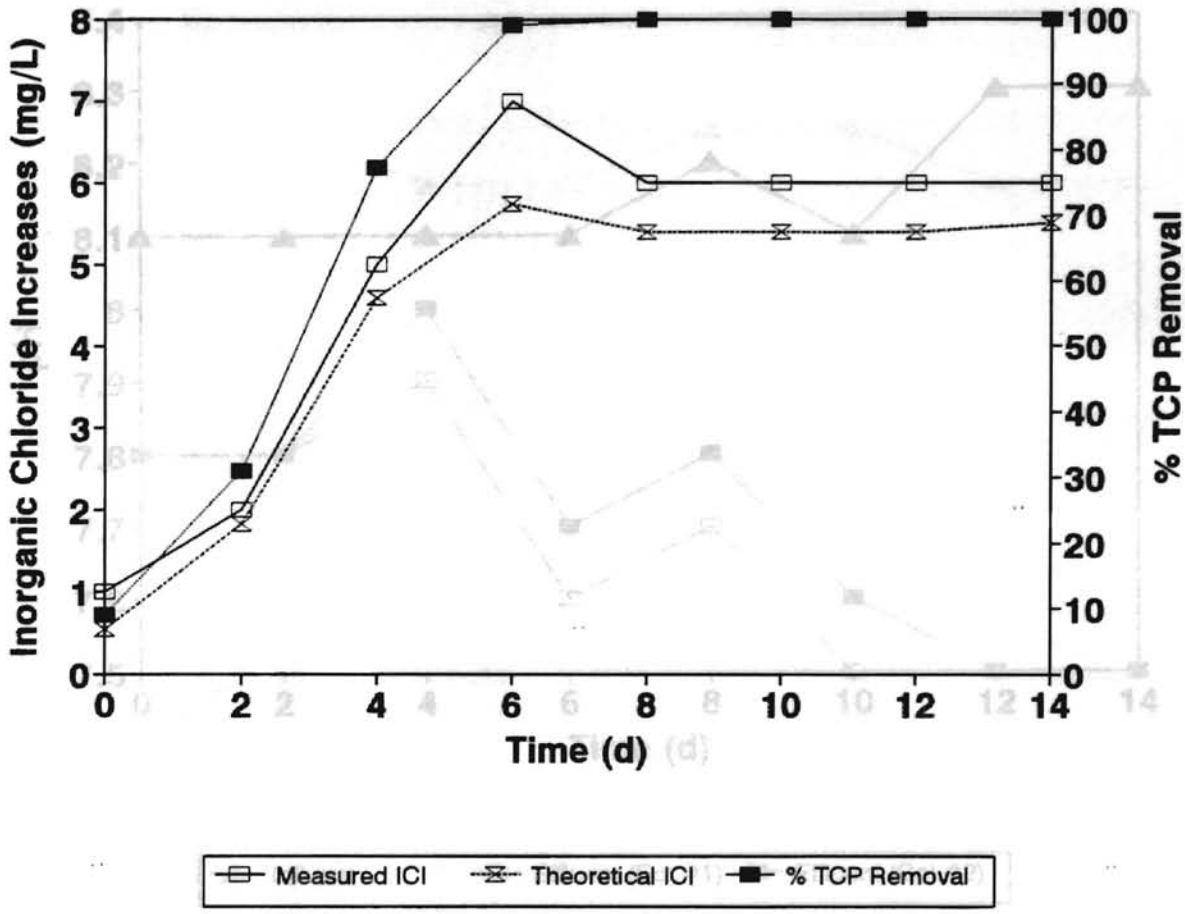


Figure 40. Final column study, column #2 -- Comparison of %TCP removal, measured and theoretical inorganic chloride releases.

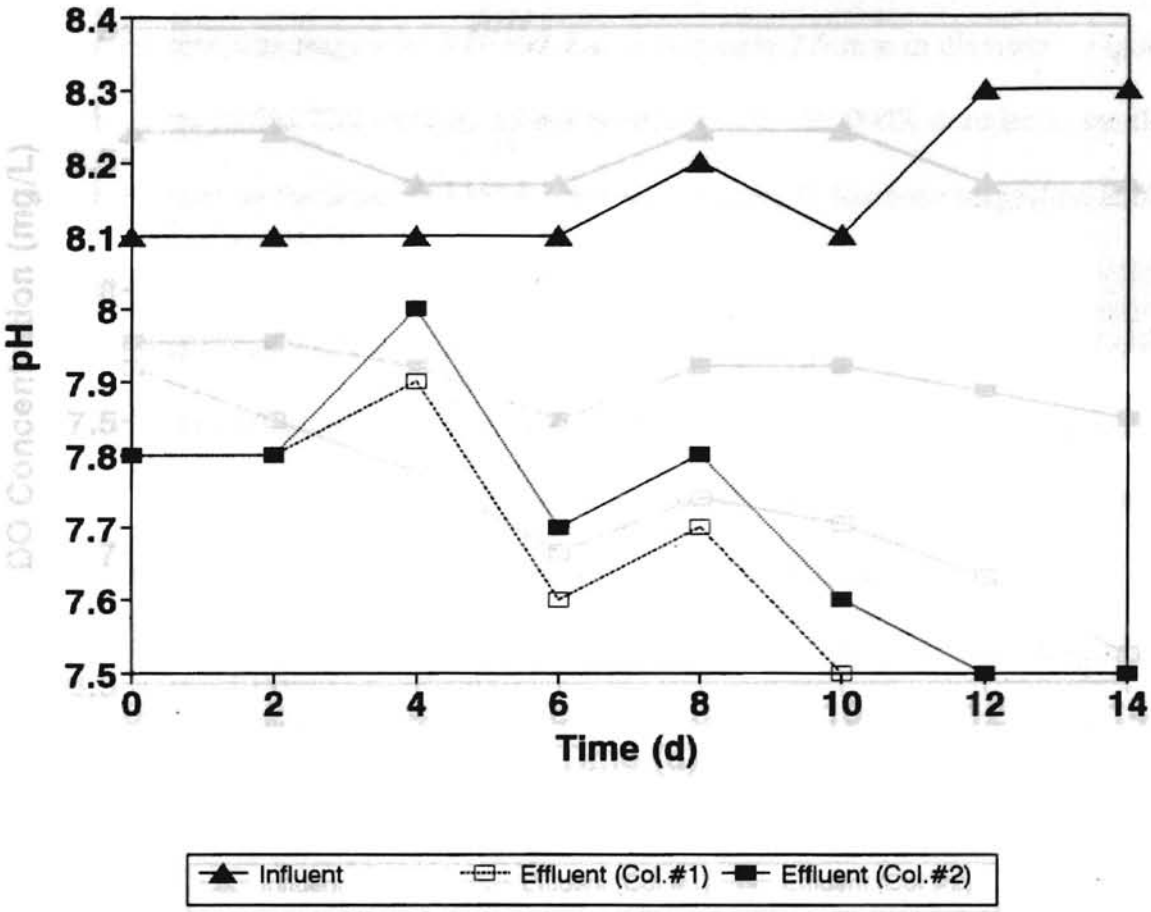


Figure 41. Comparison of pH -- columns #1 and #2.

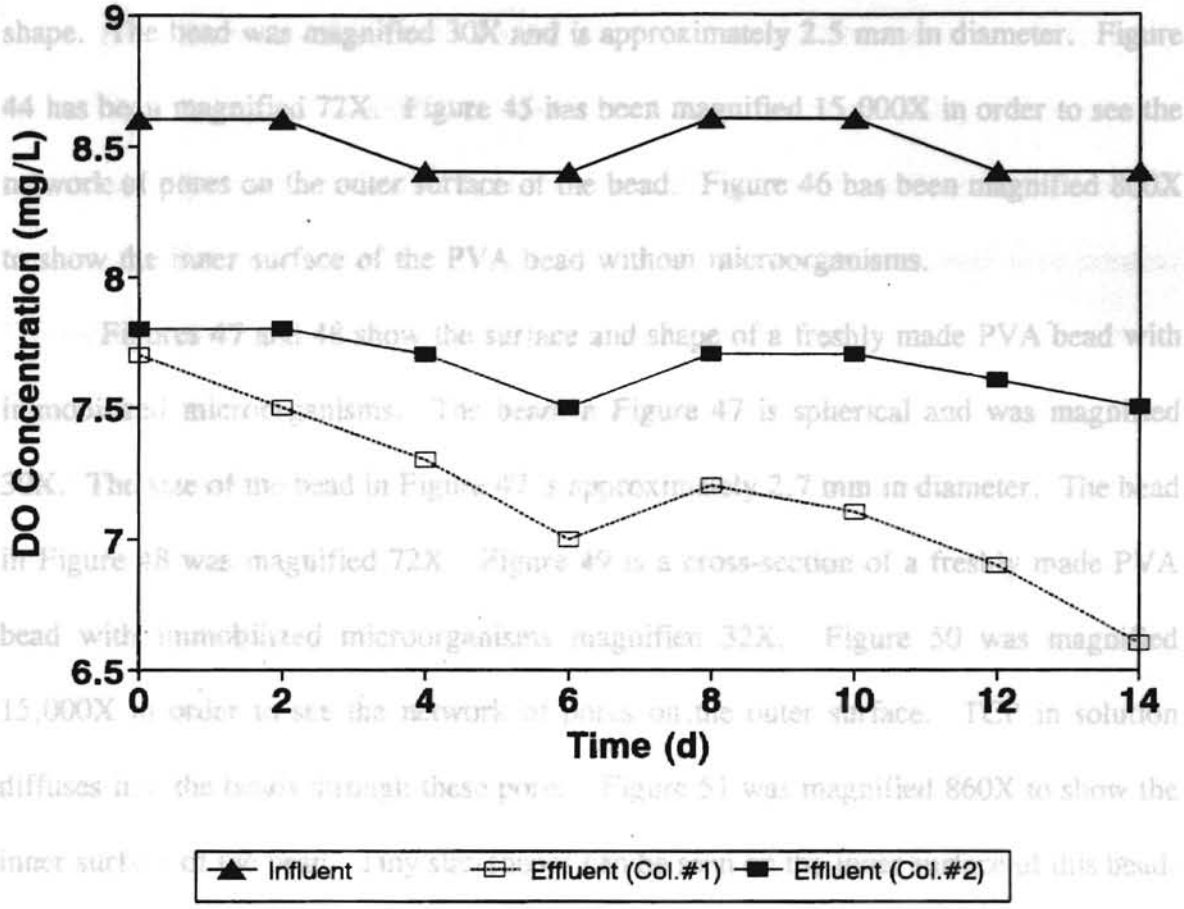


Figure 42. Comparison of DO -- columns #1 and #2.

Micrographs of PVA Beads and Immobilized Cells

Figures 43 and 44 show the surface and shape of a freshly made PVA bead without microorganisms. The outer surface of the bead is smooth and is spherical in shape. The bead was magnified 30X and is approximately 2.5 mm in diameter. Figure 44 has been magnified 72X. Figure 45 has been magnified 15,000X in order to see the network of pores on the outer surface of the bead. Figure 46 has been magnified 860X to show the inner surface of the PVA bead without microorganisms.

Figures 47 and 48 show the surface and shape of a freshly made PVA bead with immobilized microorganisms. The bead in Figure 47 is spherical and was magnified 30X. The size of the bead in Figure 47 is approximately 2.7 mm in diameter. The bead in Figure 48 was magnified 72X. Figure 49 is a cross-section of a freshly made PVA bead with immobilized microorganisms magnified 32X. Figure 50 was magnified 15,000X in order to see the network of pores on the outer surface. TCP in solution diffuses into the beads through these pores. Figure 51 was magnified 860X to show the inner surface of the bead. Tiny star-shapes can be seen on the inner surface of this bead. The star-shapes were magnified to 11,000X in Figure 52 and further magnified to 20,000X in Figure 53. The crosslinking of the polymer and the pores can be seen behind the star-shapes. The star-shapes are approximately 2.2×10^{-3} mm to 4.0×10^{-3} mm in length. These star-shapes were not seen in the PVA beads without microorganisms. It was speculated that the star-shapes were bacteria that aggregated together, because they were only visible in the beads containing immobilized bacteria. It is further speculated that the method and chemicals used for immobilization caused the microorganisms to

aggregate together, forming star-shapes. The immobilization process was conducted twice, with the same results. Figure 54 shows the mixed culture of microorganisms that were immobilized in the PVA beads. Figure 54 is magnified 20,000X.

PVA-immobilized cells were used in a batch experiment and in a column study for 45 days. Figure 55 shows a whole bead from the batch study, 45 days old, magnified 30X. Figure 56 shows a cross-section of an immobilized bead magnified 26X from the batch experiment after 45 days. The outer surface has become more porous. The inner area appears to have more channels and pockets than the freshly made beads. Figure 57 is a cross-section of an immobilized bead magnified 32X from the batch experiment in which a pocket of spherical shaped cells has been enlarged by 660X in Figure 58. The same pocket was further enlarged 3600X in Figure 59 to better show the cells, pores, and cross-linking. The organisms are approximately 1.4×10^{-3} mm to 4.0×10^{-3} mm in diameter.

Figure 60 shows the whole bead, 45 days old, magnified 32X, from the column study. The bead is not as smooth as the bead from the batch experiment. Figure 61 is a cross-section of a bead from the column study that was magnified 32X. It appears to have more channels and pockets than the freshly made beads. Figure 62 is an enlargement of the designated area on Figure 61. It was magnified 3600X and shows cells, cross-linking, and pores inside the bead. The cells are approximately 8.3×10^{-4} mm to 2.2×10^{-3} mm in diameter. There were no star-shaped bacteria seen in the beads after 45 days. Figure 63 is the outer surface of the bead magnified 11,000X showing that microorganisms are attached to the outer areas of the beads.

Summary

The purpose of the initial feasibility study was to evaluate PVA beads as a permeable barrier media. The two aspects of using the PVA beads as a permeable barrier were assessed: 1) physical characteristics of the beads; and, 2) ability as a biological carrier system. The results of the preliminary studies, batch studies, and column studies that were conducted for this research are summarized and evaluated in Table 5.

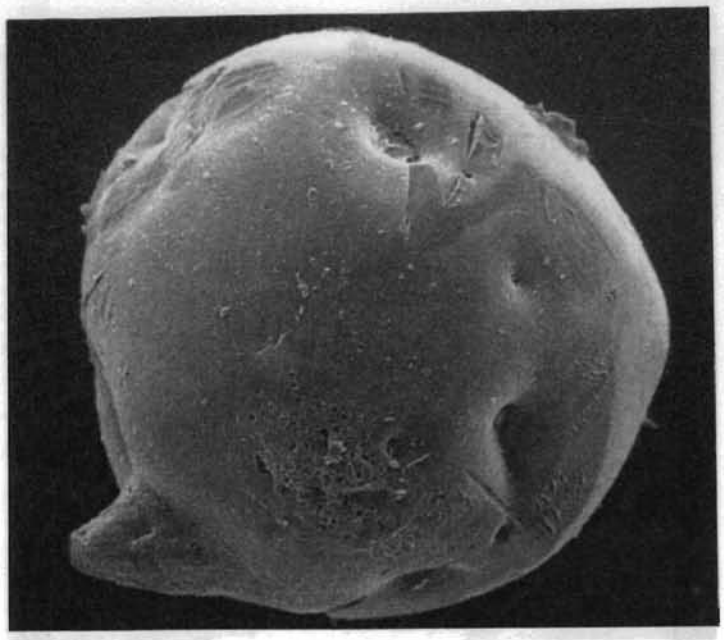


Figure 43. PVA bead without microorganisms (30X).

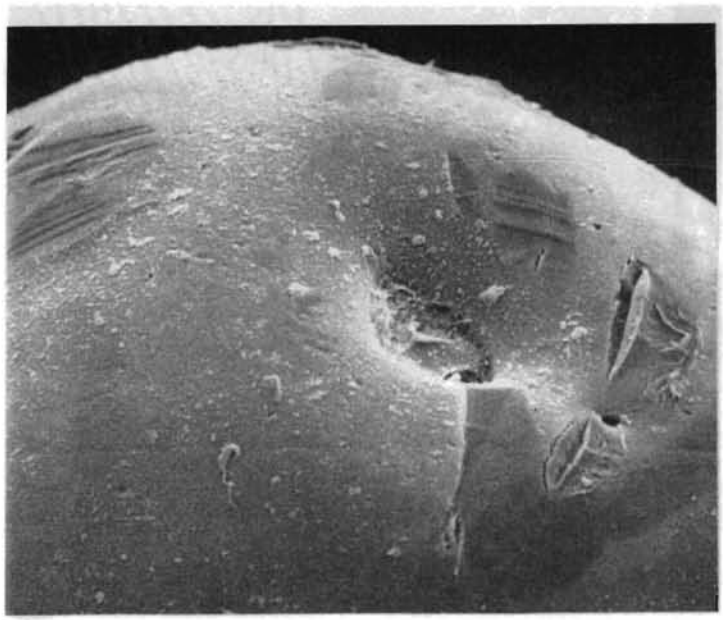


Figure 44. PVA bead without microorganisms (72X).

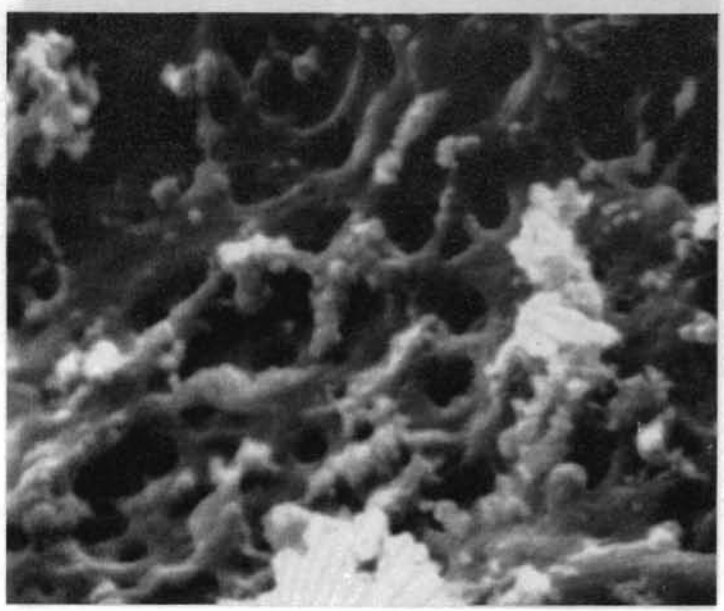


Figure 45. PVA bead without microorganisms - outer surface (15,000X).

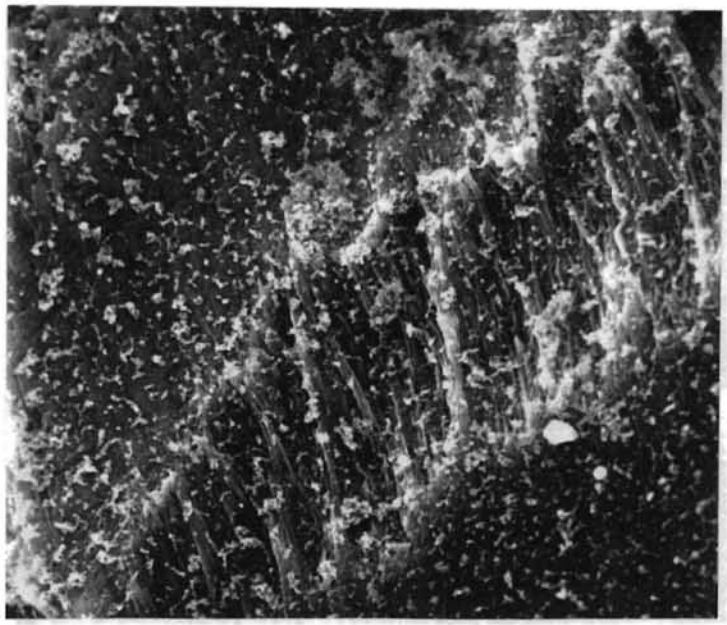


Figure 46. PVA bead without microorganisms - inside surface (860X).

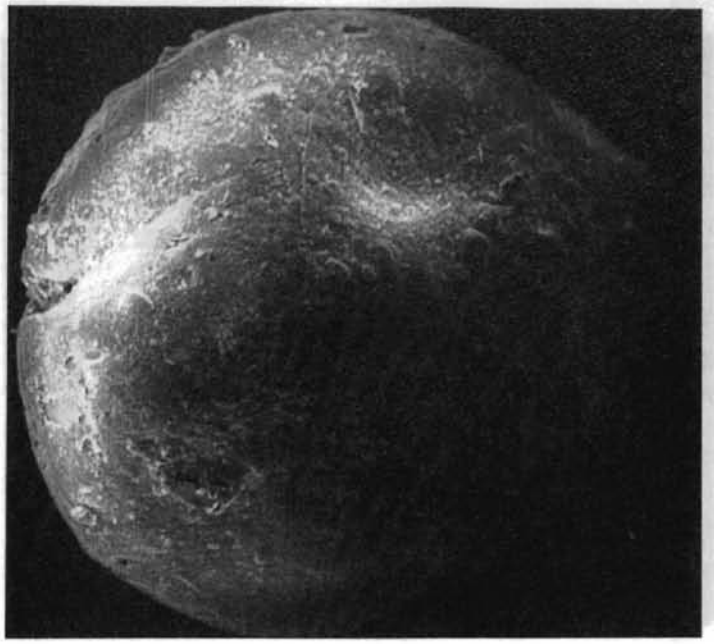


Figure 47. PVA bead with microorganisms, 2 days old (30X).

Figure 48. PVA bead with microorganisms, 2 days old (72X).

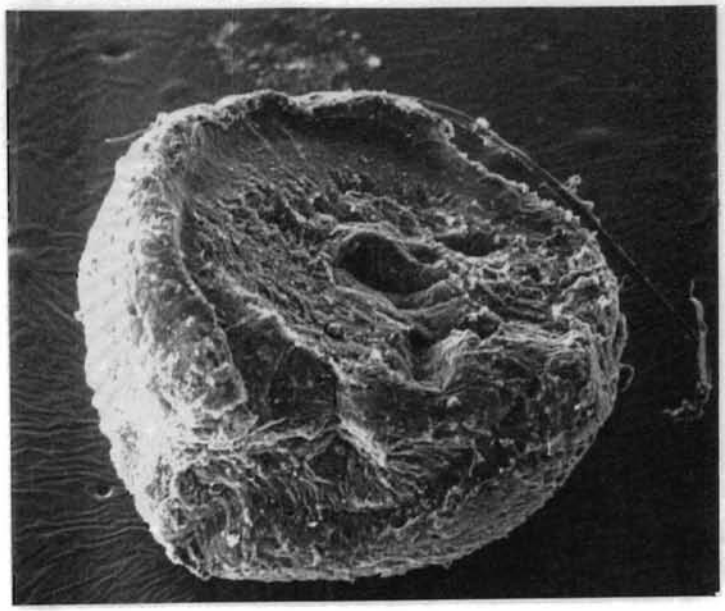


Figure 49. Cross-section of PVA bead with microorganisms, 2 days old (32X).

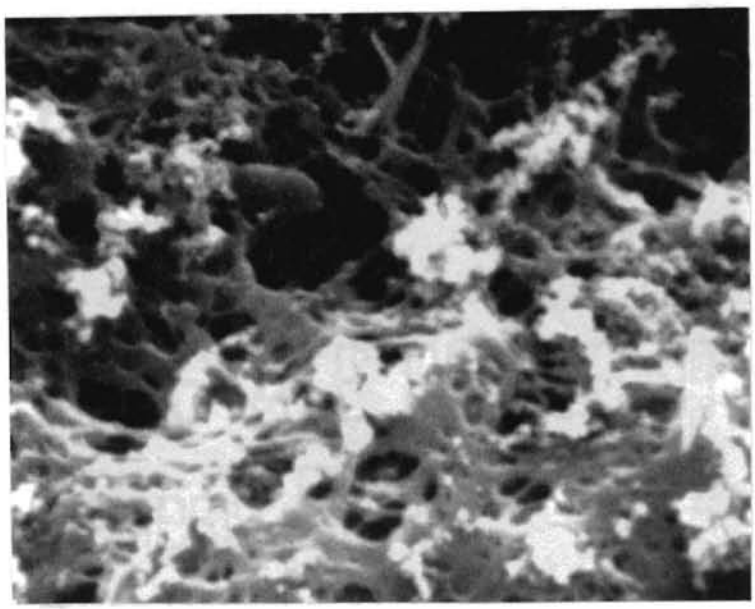


Figure 50. Surface of PVA bead with microorganisms, 2 days old (15,000X).

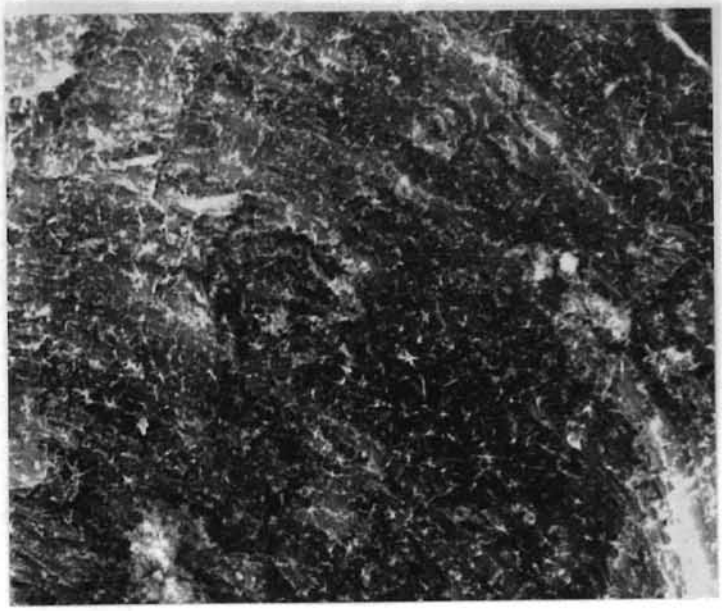


Figure 51. Inner surface of PVA bead with microorganisms, 2 days old (860X).

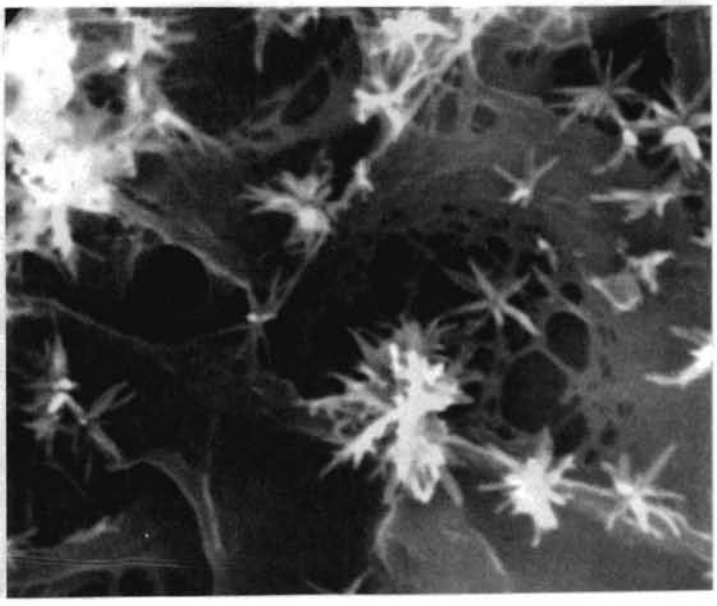


Figure 52. Inside of PVA bead with microorganisms shows star-shaped clusters, 2 days old (11,000X).

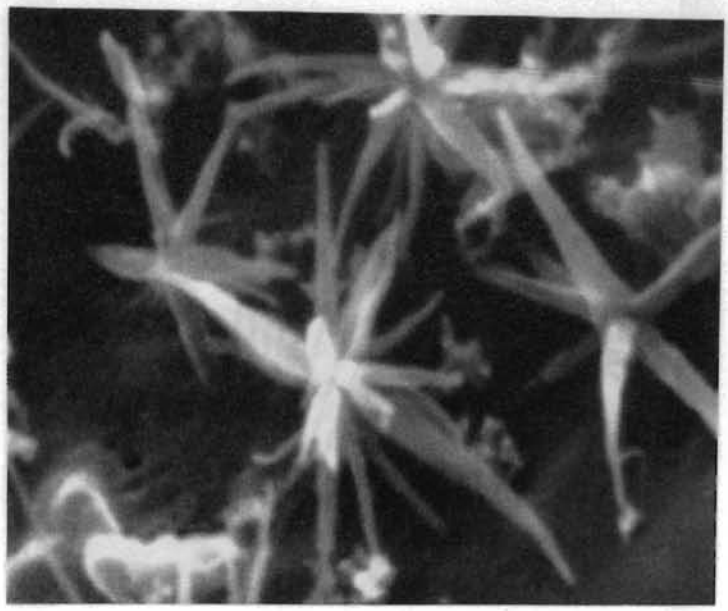


Figure 53. The same star-shaped clusters as shown in Figure 47 enlarged (20,000X).

Figure 54. Centrifuged biomass prior to immobilizing into PVA beads (20,000X).

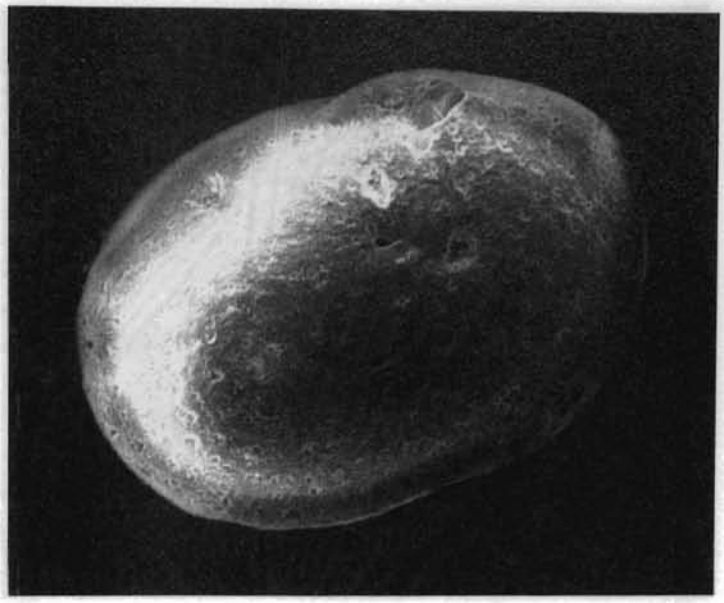


Figure 55. PVA bead with microorganisms from batch experiment, 45 days old (30X).

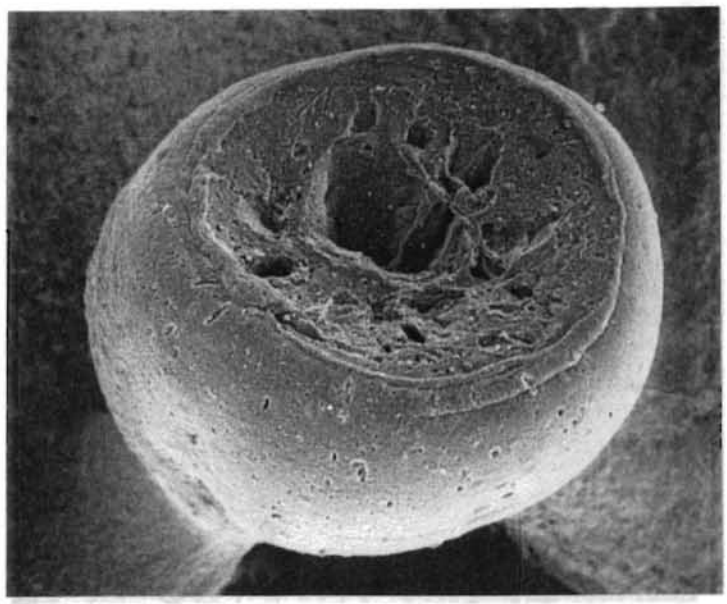


Figure 56. Cross-section of PVA bead with microorganisms from batch experiment, 45 days old (26X).

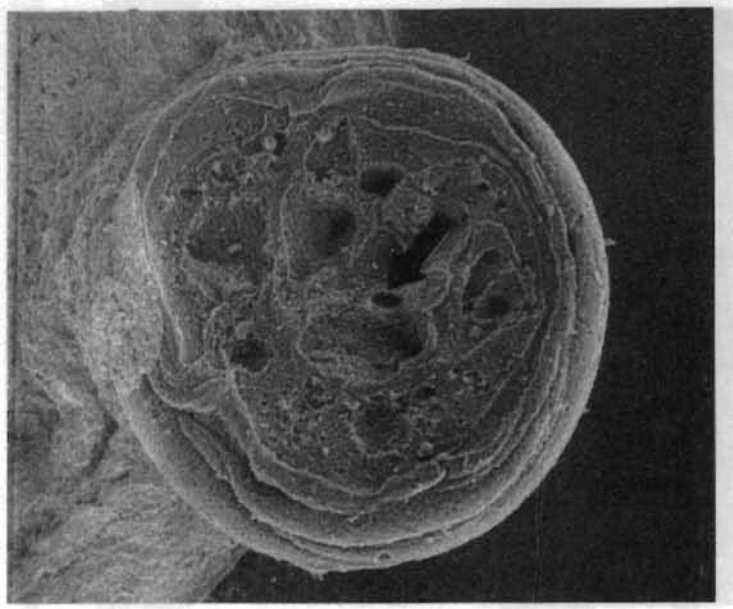


Figure 57. Cross-section of PVA bead with microorganisms in which the designated area is a pocket of cells (32X).

Figure 58. Pocket of cells enlarged from Figure 57, 45 days old (660X).

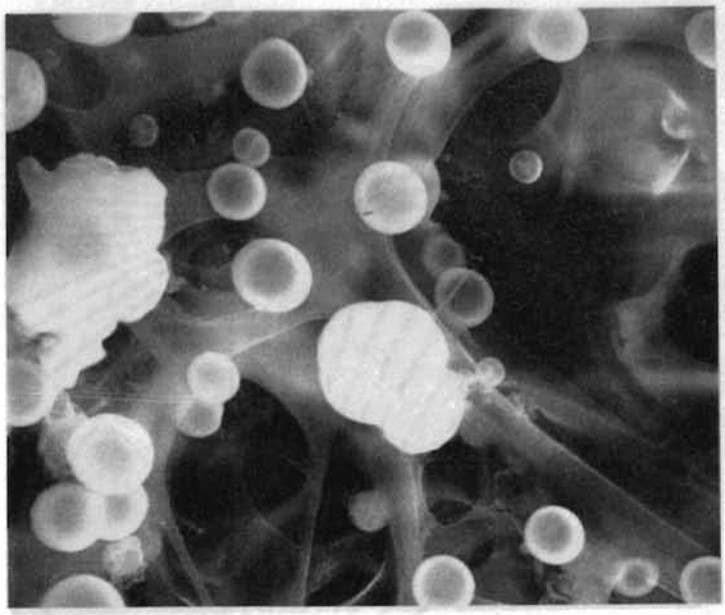


Figure 59. A further magnification of the same cells as shown in Figure 58, (3600X).

Figure 60. PVA bead with microorganisms from the column study, 45 days old (32X).

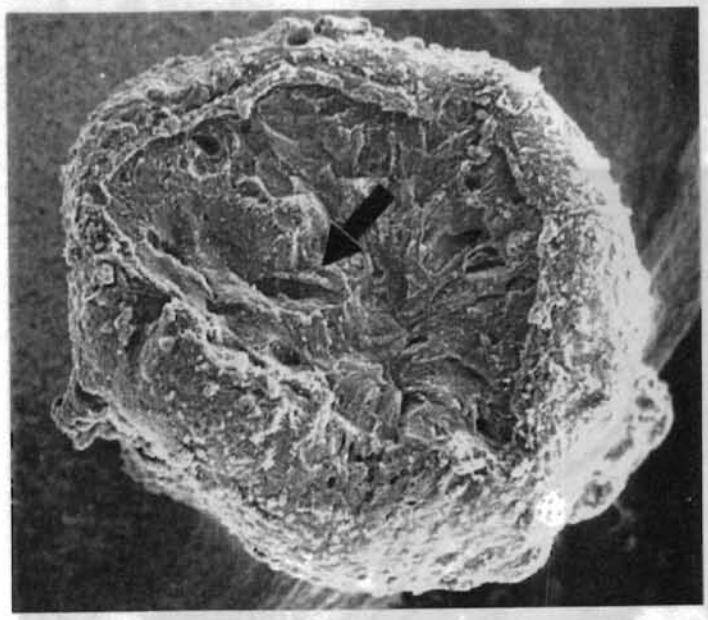


Figure 61. Cross-section of PVA bead with microorganisms from the column study, 45 days old (32X).

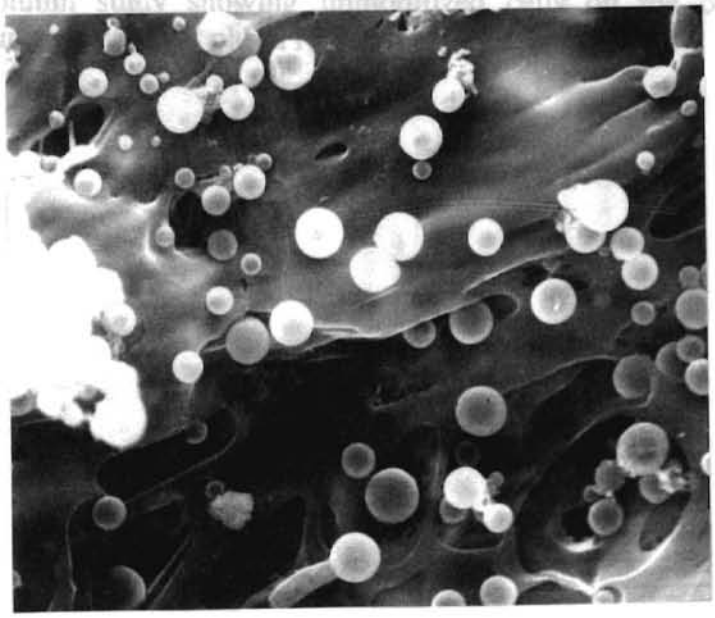


Figure 62. Population of cells inside the PVA bead from the column study, enlarged from designated area in Figure 61, 45 days old (3600X).

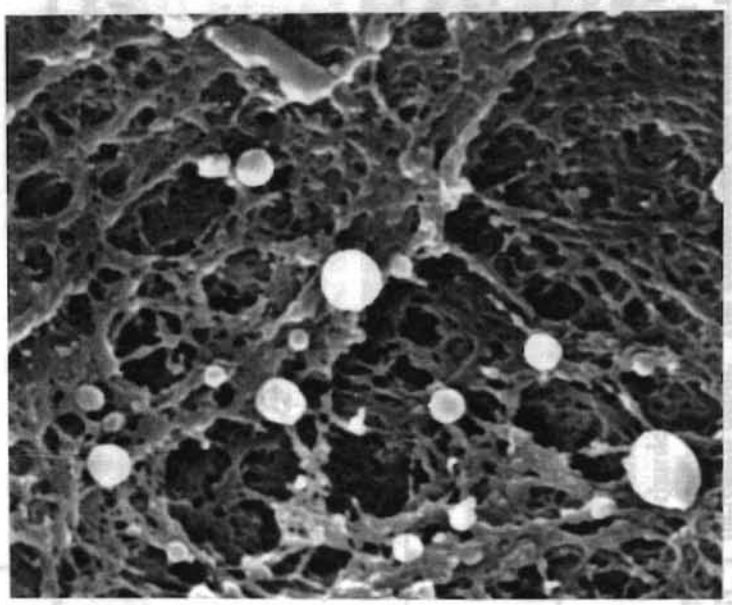


Figure 63. PVA bead with microorganisms from the column study showing immobilized cells on the outer surface, 45 days old (11,000X).

TABLE 5.

SUMMARY OF RESULTS

Experiment/ Study	Results	Evaluation
(1) Porosity test	▪ specific gravity @ 22°C - beads 1.008 ▪ density @ 22°C - beads 0.9869 g/cm^3 - water 0.9793 g/cm^3 - sand 1.6 g/cm^3 ▪ porosity percent - beads 25% - sand 30%	The 25% porosity of the bed of beads was comparable to aquifers composed of gravel and sand and would not impede the flow of groundwater. The density of the beads was greater than water, which eliminated flotation problems.
(2) Falling head permeameter test	▪ permeable coefficient (k) - beads 0.1425 cm/s - sand 0.0162 cm/s	The permeability coefficient for the bed of beads was comparable to values for course sand to fine gravel and would allow the flow of water through the "bio-trench" at a compatible rate.
(3) Compression test	▪ deformation percent of beads 48% ▪ compressibility index (C_c) - beads 4.08 X 10^{-3} m^2/kN - sand 2.87 X 10^{-5} m^2/kN	The bed of beads had similar compressibility properties as a very soft clay. The bed of beads compressed 48% with an overburden pressure equivalent to that found at the bottom of a 40 ft ditch. Its porosity and permeability could be effected with increased depth. The compressed bed returned to its original size within 24.0 hours with no apparent permanent deformation.

TABLE 5 - *Continued.*

Experiment/ Study	Results	Evaluation
(4) Diffusion study	▪ diffusion coefficient (D) 3.1 X 10^{-6} cm^2/s	TCP was able to diffuse into the PVA bead at a rate similar to that in other bead materials used to immobilize bacteria.
(5) Adsorption study	▪ adsorption capacity (K) 5.01 X 10^{-15} L/g ▪ adsorption intensity (1/n) 11.1	The adsorption capacity was so low that virtually no physical removal of TCP by adsorption took place. Therefore any TCP removal was attributed to biodegradation.
(6) Kinetic study	▪ Free Cells substrate use rate (r_{su}) 1.11 mg/L· hr growth rate (r_g) 3.9 mg VSS/L· hr ICl released 8.0 mg/L ▪ Immobilized Cells substrate use rate (r_{su}) 1st) 0.14 mg/L· hr 2nd) 0.47 mg/L· hr 3rd) 2.0 mg/L· hr ICl released 7.0 mg/L	The substrate utilization rate and growth rate of the free cells showed that the bacteria were active, growing, and utilizing the TCP as substrate prior to immobilization. The immobilized cells utilized TCP as a substrate. The ICl increases tend to support that dehalogenation of TCP occurred. GC-MS analyses on samples taken from the immobilized cell study further support the dehalogenation of TCP. The DO consumption verified that it was an aerobic system.

TABLE 5 - *Continued.*

Experiment/ Study	Results	Evaluation
(7) Tracer studies	▪ Initial Tracer Study tracer study: step/F curve flowrate (Q): 0.973 mL/min dispersion (D/μL): 0.2 HRT: 111.1 min % Br recovery: 98% ▪ Final Tracer Study *Column #1* tracer study: pulse/E curve flowrate (Q): 1.05 mL/min dispersion (D/μL): 0.1933 HRT: 39 min % Br recovery: 99.5% *Column #2* tracer study: pulse/E curve flowrate (Q): 1.02 mL/min dispersion (D/μL): 0.0632 HRT: 98 min % Br recovery: 96.5%	The column in the initial column study had a large amount of dispersion taking place. The F-curve had three slopes which indicated that the flow traveled through three different mediums (lower bed of sand, beads, and upper bed of sand). The inflection points on the curve represented the interfaces between the different mediums. The first maximum slope occurred at the point where θ equals 1.0 and the second maximum slope occurred at the point where θ equals 1.75, and the third maximum slope occurred at the point where θ equals 2.25. Column #1 displayed a large amount of dispersion. Bromide concentrations showed up at the effluent port immediately and peaked at the point where θ equals 1.0. The E curve had a broad base indicating a large amount of dispersion similar to a completely mixed reactor. Column #2 displayed an intermediate amount of dispersion. The E curve showed that a walling effect took place and then a sharp peak occurred at the point where θ equals 1.1. The sharp peak was similar to a plug flow reactor.

TABLE 5 - *Continued.*

Experiment/ Study	Results	Evaluation
(8) Column Studies	▪ Initial Column Study (10.0 cm of beads with sand) % removal 100% within 14 days ICl increase 160 mg/L to 167 mg/L pH decrease 8.3 to 7.8 DO decrease 8.6 mg/L to 2.5 mg/L ▪ Final Column Study *Column #1* (8.0 cm of beads) % TCP removal 100% within 10 days ICl increase 158 mg/L to 164 mg/L pH decrease 8.3 to 7.5 DO decrease 8.6 mg/L to 6.6 mg/L *Column #2* (20.0 cm of beads) % TCP removal 100% within 8 days ICl increase 158 mg/L to 164 mg/L pH decrease 8.3 to 7.5 DO decrease 8.6 mg/L to 7.5 mg/L	The initial column study had 100% removal within 14 days. An increase in ICl, with a concomitant decrease in pH, tends to support dehalogenation of TCP. A GC-MS analysis further supported dehalogenation of TCP. Column #1 had 100% removal of TCP within 10 days. Thereafter, it was as efficient as column #2 in removing TCP. An increase in ICl, with a concomitant decrease in pH, tends to support dehalogenation of TCP. Column #2 had 100% removal within 8 days. An increase in ICl, with a concomitant decrease in pH, tends to support dehalogenation of TCP.

TABLE 5 - *Continued.*

Experiment/ Study	Results	Evaluation
(9) DAPI stain	▪ Organisms fluoresced under the epifluorescence microscope	The thin sections of beads containing immobilized bacteria fluoresced under the epifluorescence microscope. This verified that organisms containing DNA were immobilized within the beads prior to setting up the column studies.
(10) Electron micrographs	▪ bacteria population ▪ immobilization sites ▪ morphology ▪ colonies ▪ physical changes to beads	The micrographs verified that the organisms were immobilized within the beads. The centrifuged sludge, prior to immobilization, contained rod-shaped bacteria. The bacteria were speculated to be star-shaped after immobilization. The bacteria were spherical after 45 days. The spherical shaped bacteria were found congregated in pockets within the beads. There were some bacteria on the surface of the beads taken from the initial column study. No colonies were seen in the micrographs. The beads containing immobilized bacteria appeared to be more porous and the channels and pockets inside the beads appeared larger after 45 days of operation. The beads appeared to be resilient, firm, and structurally sound after 45 days of operation.
(11) GC-MS analysis	▪ mineralization of TCP	The GC-MS analysis for the influent and effluent samples obtained from the initial column study showed that no intermediates developed from the removal of 10.0 mg/L TCP. This supported complete dehalogenation of TCP.

CHAPTER V

CONCLUSIONS

Based on this investigation, the results demonstrated that PVA-immobilized cells would be a successful permeable barrier media for use in a bio-trench to remove TCP in situ from groundwater. Other findings are were follows:

1. The concept of a bio-trench using PVA-immobilized cells as a permeable barrier media to remove TCP from groundwater appears to be feasible as demonstrated in the initial column study. The column was composed of a bed of PVA-immobilized cells situated between layers of sand. The column was designed to simulate a bio-trench system. The column had 100% removal of TCP within 14 days of operation and continued with the same efficiency for the remainder of the 45 day experiment.

2. A comparison of removal efficiencies between two columns of varied sizes containing PVA-immobilized cells demonstrated the effect of HRTs. It took 8 days for the 20 cm column to achieve 100% removal of TCP and 10 days for the 8 cm column to achieve 100% removal. But, once the 100% removal was reached there was no difference in efficiency thereafter. Both columns continued to have 100% removal.

3. Acclimated bacteria were able to use TCP as their sole carbon source, as evidenced by the free cells and immobilized cells biodegrading TCP in batch and column experiments.

4. Batch studies showed that immobilized cells needed time to overcome effects of immobilization, such as low pH conditions. The immobilized cells recovered from the effects within only 96 hours and were then capable of biodegrading 10.0 mg/L of TCP within 5.0 hours.

5. Dehalogenation of TCP by the PVA-immobilized cells in the batch and column studies was evidenced by the chloride increases and pH decreases. Dehalogenation of TCP was further supported by GC-MS analysis.

6. After 45 days of operation, the beads appeared to be resilient, firm, and structurally sound. The micrographs of the beads showed them to be more porous. The channels and pockets within the beads appeared larger.

7. Scanning electron micrographs showed changes in the morphology of the bacteria. Rod shaped bacteria were found in the centrifuged biomass prior to immobilization. Star-shapes were found inside PVA-beads within two days after the immobilization process. The star-shapes were speculated to be bacteria since they were only found in those beads in which centrifuged biomass was immobilized. The star-shapes were not found in the plain beads. After 45 days of operation in a batch study and a column study spherical bacteria were found inside the beads.

8. It was observed that beads made with PVA (MW 88,000) were more rubber-like and had greater elasticity than beads made with higher molecular weights.

9. Data obtained from the diffusion study were applied to the shrinking core model (SCM). It was determined that the diffusion of TCP into PVA beads was 3.1 X 10^{-6} cm^2/s. This diffusion value was similar in comparison to rates of diffusion into other

bead materials.

10. A bed of PVA beads was characterized as to its porosity, permeability, and compressibility. The bed of beads had properties that were comparable to soils. Its compressibility was similar to a very soft clay, and porosity and permeability were comparable to a coarse sand and a fine gravel. Therefore, the beads used as a permeable barrier would not impede the flow of groundwater.

11. The compressibility study showed that the bed of PVA beads had a 48% deformation from the overburden pressure at a depth of 40 feet. Deformation could effect the flow of groundwater at lower depths.

12. The adsorption capacity of PVA beads was virtually non-existent. The adsorption capacity (K) of the beads was 5.01×10^{-15} and the adsorption intensity (1/n) was 11.1. The very low K value and high 1/n value showed that minimal physical removal was attributed to adsorption.

CHAPTER VI

SUGGESTIONS FOR FUTURE STUDY

Based on the findings of this study, several suggestions are presented for future studies involving using the PVA-immobilized cells for removal of groundwater pollutants:

1. Conduct batch and column studies to evaluate the removal of other pollutants including tetrachlorophenol and pentachlorophenol.

2. Conduct the same batch and column experiments at ground depths that would be expected in its application as a bio-trench to see effects under different pressures.

3. Conduct same experiments at different flow rates and different concentrations to see the removal efficiency and effect of toxicity to the immobilized cells.

4. Conduct further studies on the star-shaped phenomena that occurs upon immobilization of the cells using the PVA boric-acid method.

5. Conduct diffusion studies on aged beads containing bacteria to see effect that immobilization and microbial activity would have on diffusion. The microorganisms would need to be destroyed prior to the diffusion study to eliminate any uptake of TCP by the bacteria.

6. Evaluate different methods to measure growth rate of bacteria within the beads.

7. Conduct further studies on the oxygen consumption of the immobilized bacteria.

8. Compare the PVA beads containing immobilized bacteria to other permeable barrier mediums.

9. Conduct a field study using PVA immobilized cells as a permeable barrier medium in a "bio-trench."

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APPENDIX - A

PROPERTIES OF CHLOROPHENOLS

TABLE 6.

INFORMATION ON THE IDENTITY OF CHLOROPHENOL CONGENERS

CAS Number	Common Name	Abbreviation	Molecular Formula	Common Synonyms	Trade Names
95-57-8	2-monochlorophenol	2-MCP	C_6H_5ClO	o-chlorophenol ortho-chlorophenol 1-chloro-2-hydroxybenzene	
120-83-2	2,4-dichlorophenol	2,4-DCP	$C_6H_4Cl_2O$	NCI-C55345	
88-06-2	2,4,6-trichlorophenol	2,4,6-TCP	$C_6H_3Cl_3O$	NCI-C02904	Dowicide 2S; Omal; Phenachlor
58-90-2	2,3,4,6-tetrachlorophenol	2,3,4,6-TeCP	$C_6H_2Cl_4O$		Dowicide 6
	pentachloride	PCP	C_6Cl_5OH		

Source: WHO, 1989.

TABLE 7.

PHYSICAL AND CHEMICAL PROPERTIES OF CHLOROPHENOLS

Compound	Relative Molecular Mass	Density	Boiling Point (°C at 760mm)	Melting Point (°C at 760mm)	Flash Point (°C)	Vapor Pressure (mm) (temperature)	Log n-octanol/water partition coefficient
2-MCP	128.56	1.2634	174.9	9.0	63.9	1(12.1°C)	2.15
2,4-DCP	163.0	1.28	210.0	45.0	62.0	1(76.5°C)	3.06
2,4,6-TCP	197.45	1.49	246.0	69.5	113.9		
2,3,4,6-TeCP	231.98	1.6	150.0 (15 mm)	70.0		1(100°C)	4.10
PCP	266.35	1.978	309-310	190-191		0.12(100°C)	5.01

Source: WHO, 1989.

APPENDIX - B

TABLES

TABLE 8.

GROUNDWATER ANALYSIS

Parameter	Concentration	EPA Method 40 CFR Part 136
Specific Conductance	1045.66 μmhos/cm	120.1
pH	7.9 std unit	150.1
Alkalinity (total)	237.3 mg/L	310.2
Solids, (total dissolved)	515.1 mg/L	160.1
Nitrite-Nitrate as N	0.5 mg/L	353.2
Hardness (total)	106.9 mg/L	130.1
Chloride	143.9 mg/L	325.2
Sulfate	32.9 mg/L	375.2
TOC	1.5 mg/L	--

TABLE 9.

COMPRESSIBILITY DATA

Stress (tons/ft^2)	Time (min)	Consolidation (in)	Trench Depth (ft)	Cumulative Strain (%)
0.00	0.0	0.351	0	0
0.05	5.0	0.329	1.6	6.3
0.06	11.0	0.318	1.9	9.7
0.08	18.0	0.296	2.6	15.7
0.11	24.0	0.273	3.6	22.2
0.17	30.0	0.251	5.5	28.2
0.23	35.0	0.245	7.5	30.2
0.38	39.0	0.238	12.3	32.2
0.51	42.0	0.233	16.5	33.3
0.63	60.0	0.217	20.4	37.9
0.89	65.0	0.211	28.9	39.9
1.02	91.0	0.190	33.1	45.9
1.28	107.0	0.181	41.5	48.1
1.28	200.0	0.173	41.5	50.7
1.28	300.0	0.170	41.5	51.6
1.28	400.0	0.166	41.5	52.7
1.28	500.0	0.162	41.5	53.8

TABLE 10.

ADSORPTION DATA
Copper Screen

Time (hrs)	TCP (mg/L)
0	10.0
3	10.0
7	10.0
12	10.0
24	10.0

TABLE 11.

ADSORPTION DATA
Aquifer Sand

Time (hrs)	TCP (mg/L)
0	10.0
3	9.6
7	9.8
12	9.9
24	10.0

TABLE 12.

EQUILIBRIUM STUDY DATA
PVA Beads and TCP

Time (min)	TCP (mg/L)
0	20.0
2	18.9
6	17.2
15	15.9
30	15.5
90	14.9
120	14.7
180	14.5
420	14.0
720	14.0
1440	14.0

TABLE 13.

FREUNDLICH ISOTHERM DATA
PVA Beads

$C_{initial}$ (mg/L)	C_{final} (mg/L)	X (mg)	M (g)	q=X/M (mg/g)	Log q	Log C
24.0	24.000	0	0	0	0	0
24.0	21.361	2.639	1.0	2.64	0.42	1.33
24.0	19.147	4.853	5.0	0.971	-0.01	1.28
24.0	18.606	5.394	10.0	0.539	-0.27	1.27
24.0	17.918	6.082	15.0	0.405	-0.39	1.25
24.0	17.925	6.075	20.0	0.304	-0.52	1.25

TABLE 14.

DIFFUSION STUDY DATA

Time (min)	TCP Concentration (mg/L)
0	10.0
2	9.0
4	8.5
6	8.0
8	8.0
15	8.0
20	7.5
30	6.75
45	6.6
90	5.5
120	5.5
180	5.5
300	5.5

TABLE 15.

SHRINKING CORE MODEL
Diffusion Data

Time (t) (min)	TCP Concentration (C) (mg/L)	X	F(x)	∫ Cdt (mg/L·min)
0	10.0	0	0.000	0
2	9.0	0.222	0.016	19.0
4	8.5	0.333	0.047	36.5
6	8.0	0.444	0.087	53.0
8	8.0	0.444	0.087	69.0
15	8.0	0.444	0.087	125.0
20	7.5	0.555	0.146	164.0
30	6.75	0.722	0.284	235.0
45	6.6	0.755	0.321	335.1
90	5.5	1.000	1.000	607.4
120	5.5	1.000	1.000	772.4
180	5.5	1.000	1.000	1102.4
300	5.5	1.000	1.000	1762.4

TABLE 16.

BATCH STUDY DATA
Free Cells

Time (hrs)	TCP (mg/L)	Cl^- Theoretical (mg/L)	Cl^- Measured (mg/L)	VSS (mg/L)
0	10.0	550.00*	550*	888
3	7.5	551.34**	552	897
6	4.5	552.96	556	905
9	0	555.39	558	923
14	0	555.39	559	942
22	0	555.39	558	989

* This represents the background Cl^- concentration in the samples.

** 0.539 mg/L Cl^- is released per 1 mg/L TCP dehalogenated; therefore, 10 mg/L - 7.5 mg/L = 2.5 mg/L TCP dehalogenated and 2.5 X 0.539 = 1.34 mg/L Cl^- increase expected.

TABLE 17.

TCP BIODEGRADATION
Immobilized Cells

Time (hrs)	First Feeding Period TCP (mg/L)	Second Feeding Period TCP (mg/L)	Third Feeding Period TCP (mg/L)
0	10.0	10.0	10.0
1	9.75	9.5	7.5
3	9.5	8.0	4.0
5	9.0	7.0	0.0
8	8.5	5.0	0.0
12	8.0	2.8	--
18	7.5	0.9	--
24	6.8	0.0	--
48	2.6	--	--
72	0.0	--	--

TABLE 18.

BATCH STUDY DATA
Immobilized Cells

Time (hrs)	TCP (mg/L)	Cl^- Theoretical (mg/L)	Cl^- Measured (mg/L)	DO (mg/L)
0	10.0	160.00*	160.00*	8.6
1	7.5	161.34**	163.00	8.2
3	4.0	163.23	166.00	6.9
5	0	165.39	167.00	6.0
8	0	165.39	167.00	5.4
12	0	--	--	--
18	0	--	--	--
24	0	--	--	3.3

* This represents the background Cl^- concentration in the samples.

** 0.539 mg/L Cl^- is released per 1 mg/L TCP dehalogenated; therefore, 10 mg/L - 7.5 mg/L = 2.5 mg/L TCP dehalogenated and 2.5 X 0.539 = 1.34 mg/L Cl^- increase expected.

TABLE 19.

INITIAL TRACER STUDY DATA

Time (min)	θ	Volume (mL)	Accumulative Volume (L)	Bromide (mg/L)	F
0	0	0	0	0	0
12	0.108	12	0.0120	0	0
24	0.216	11.5	0.0235	0	0
36	0.324	12	0.0355	0	0
48	0.432	12	0.0475	0.32	0.0039
60	0.540	12	0.0595	0.35	0.0043
72	0.648	11.5	0.0710	0.37	0.0045
84	0.756	11.75	0.0827	0.95	0.0116
96	0.864	12	0.0947	7.5	0.0915
108	0.972	11.5	0.1062	14.5	0.1768
120	1.080	11.5	0.1177	19.5	0.2378
132	1.188	12	0.1297	24	0.2927
144	1.296	11.5	0.1412	30.75	0.3750
156	1.404	11.5	0.1527	35	0.4268
168	1.512	11.5	0.1642	41	0.5000
180	1.620	11.5	0.1757	43.5	0.5305
192	1.728	11.5	0.1872	43.5	0.5305
204	1.836	12	0.1992	43.5	0.5305
216	1.944	11.5	0.2107	45	0.5488
228	2.052	11.5	0.2222	46.5	0.5671
240	2.160	11.5	0.2337	47.5	0.5793
252	2.268	11.5	0.2452	52	0.6341

TABLE 19 - *Continued.*

Time (min)	θ	Volume (mL)	Accumulative Volume (L)	Bromide (mg/L	F
264	2.376	11.5	0.2567	55	0.6707
276	2.484	11.5	0.2682	60	0.7317
288	2.592	12	0.2802	65	0.7927
300	2.700	11.5	0.2917	70	0.8537
312	2.808	11.5	0.3032	73	0.8902
324	2.916	11.5	0.3147	77	0.9390
336	3.024	12	0.3267	77	0.9390
348	3.132	11.5	0.3382	77	0.9390
360	3.240	11.5	0.3497	77	0.9390

TABLE 20.

FINAL TRACER STUDY DATA
Column #1

Time (t) (min)	C (mg/L)	Ct (mg/L·min)	θ	E	θ^2	θ^2E	Volume (L)
0	0	0	0	0	0	0	0
4	0.90	3.60	0.1443	.0575	0.0208	0.0012	0.0044
8	3.75	30.00	0.2887	.2396	0.0833	0.0200	0.0087
12	9.50	114.00	0.4331	0.6069	0.1875	0.1140	0.0127
16	10.00	160.00	0.5774	0.6389	0.3334	0.2130	0.0170
20	12.50	250.00	0.7218	0.7986	0.5209	0.4160	0.0210
24	14.50	348.00	0.8661	0.9264	0.7501	0.6950	0.0252
28	17.50	490.00	1.0105	1.1181	1.0210	1.1416	0.0294
32	13.33	426.60	1.1548	0.8516	1.3336	1.1358	0.0337
36	7.00	252.00	1.2991	0.4472	1.6878	0.7548	0.0381
40	5.00	200.00	1.4435	0.3194	2.0838	0.6656	0.0424
44	4.75	209.00	1.5879	0.3035	2.5213	0.7652	0.0467
48	2.90	139.20	1.7322	0.1853	3.0006	0.5559	0.0511
52	2.70	140.40	1.8766	0.1725	3.5215	0.6075	0.0553
56	1.70	95.20	2.0209	0.1086	4.0841	0.4436	0.0595
60	1.80	108.00	2.1653	0.115	4.6884	0.5392	0.0634
64	0.60	38.40	2.3096	0.0383	5.3344	0.2045	0.0676
68	0	0	2.4540	0	6.0221	0	0.0718
72	0	0	2.5983	0	6.7514	0	0.0758
Totals	108.43	3004.36		6.9275		8.2726	

TABLE 21.

FINAL TRACER STUDY DATA
Column #2

Time (min)	C (mg/L)	Ct (mg/L·min)	θ	E	θ^2	θ^2E	Cum. Vol. (L)
0	0	0	0	0	0	0	0
10	0.38	3.8	0.1262	0.0704	0.0159	0.0011	0.0105
20	1.80	36.0	0.2525	0.3335	0.0637	0.0213	0.0208
30	2.35	70.5	0.3787	0.4354	0.1434	0.0625	0.0310
40	2.05	82.0	0.0505	0.3798	0.2550	0.0969	0.0415
50	2.75	137.5	0.6310	0.5095	0.3985	0.2030	0.0517
60	1.70	102.0	0.7575	0.3150	0.5738	0.1807	0.0618
70	2.12	148.4	0.8837	0.3928	0.7810	0.3068	0.0723
80	6.50	520.0	1.0100	1.2044	1.0200	1.2285	0.0823
90	10.00	900.0	1.1362	1.8529	1.2910	2.3920	0.0925
100	9.00	900.0	1.2625	1.6676	1.5938	2.6579	0.1013
110	1.70	187.0	1.3887	0.3150	1.9285	0.6075	0.1118
120	1.30	156.0	1.5150	0.2409	2.2951	0.5528	0.1218
130	1.10	143.0	1.6412	0.2038	2.6936	0.5490	0.1318
140	0	0	1.7674	0	3.1239	0	0.1424
150	0	0	1.8937	0	3.5861	0	0.1525
Totals	42.75	3386.2		7.9210		8.8599	

TABLE 22.

INITIAL COLUMN STUDY DATA

Time (days)	Influent TCP Concentration (mg/L)	Effluent TCP Concentration (mg/L)
0	11.5	10.0
2	11.5	9.0
4	11.5	8.5
6	11.5	6.9
8	11.5	5.2
10	10.75	3.6
12	10.75	1.5
14	10.75	0
16	10.75	0
18	10.75	0
20	10.0	0
25	10.0	0
30	11.0	0
35	11.0	0
40	10.0	0
45	10.0	0

TABLE 23.

pH CURVE DATA

pH	Titrant 0.1 N HCl (mL)	pH	Titrant 0.1 N HCl (mL)
8.4	0	6.8	17
8.2	2.5	6.7	18
7.7	5	6.6	20
7.55	5.5	6.5	22.5
7.45	6	6.4	25
7.4	6.5	6.35	27.5
7.35	7	6.1	30
7.3	7.5	5.9	35
7.2	8	5.7	45
7.2	8.5	5.5	51
7.1	9.5	5.25	55
7	11.5	4.6	61
6.9	13.5	3	70

TABLE 24.

FINAL COLUMN STUDY DATA
Columns #1 and #2

	Influent				Effluent							
	Columns #1 and #2				Column #1				Column #2			
Time (d)	TCP (mg/L)	DO (mg/L)	ICl (mg/L)	pH	TCP (mg/L)	DO (mg/L)	ICl (mg/L)	pH	TCP (mg/L)	DO (mg/L)	ICl (mg/L)	pH
0	11.00	8.6	161	8.1	10.2	7.7	162	7.8	10.0	7.8	162	7.8
2	11.00	8.6	158	8.1	9.25	7.5	159	7.8	7.60	7.8	160	7.8
4	11.00	8.4	151	8.1	4.50	7.3	154	7.9	2.50	7.7	156	8.0
6	10.75	8.4	153	8.1	1.75	7.0	157	7.6	0.10	7.5	160	7.7
8	10.00	8.6	156	8.2	0.50	7.2	161	7.7	0	7.7	162	7.8
10	10.00	8.6	155	8.1	0	7.1	162	7.5	0	7.7	161	7.6
12	10.00	8.4	155	8.3	0	6.9	162	7.5	0	7.6	161	7.5
14	10.20	8.4	158	8.3	0	6.6	164	7.5	0	7.5	164	7.5

APPENDIX - C

CALCULATIONS

Time (min)	TCP mg/L	$X = [C_o - C]/[C_o - C_\infty]$	
0	10	$X = [10.0 - 10.0]/[10.0 - 5.5]$	0
2	9	$X = [10.0 - 9.0]/[10.0 - 5.5]$	0.222
4	8.5	$X = [10.0 - 8.5]/[10.0 - 5.5]$	0.333
6	8	$X = [10.0 - 8.0]/[10.0 - 5.5]$	0.444
8	8	$X = [10.0 - 8.0]/[10.0 - 5.5]$	0.444
10	8	$X = [10.0 - 8.0]/[10.0 - 5.5]$	0.444
12	7.5	$X = [10.0 - 7.5]/[10.0 - 5.5]$	0.555
14	6.75	$X = [10.0 - 6.75]/[10.0 - 5.5]$	0.722
16	6.6	$X = [10.0 - 6.6]/[10.0 - 5.5]$	0.755
18	5.5	$X = [10.0 - 5.5]/[10.0 - 5.5]$	1.000
20	5.5	$X = [10.0 - 5.5]/[10.0 - 5.5]$	1.000
22	5.5	$X = [10.0 - 5.5]/[10.0 - 5.5]$	1.000
24	5.5	$X = [10.0 - 5.5]/[10.0 - 5.5]$	1.000

EXAMPLE 1.

Diffusion Study

The extent of reaction (X) was determined from Equation (7):

Time (min)	TCP mg/L	$X = [C_0 - C]/[C_0 - C_\infty]$	
0	10	X = [10.0 - 10.0]/[10.0 - 5.5] =	0
2	9	X = [10.0 - 9.0]/[10.0 - 5.5] =	0.222
4	8.5	X = [10.0 - 8.5]/[10.0 - 5.5] =	0.333
6	8	X = [10.0 - 8.0]/[10.0 - 5.5] =	0.444
8	8	X = [10.0 - 8.0]/[10.0 - 5.5] =	0.444
15	8	X = [10.0 - 8.0]/[10.0 - 5.5] =	0.444
20	7.5	X = [10.0 - 7.5]/[10.0 - 5.5] =	0.555
30	6.75	X = [10.0 - 6.75]/[10.0 - 5.5] =	0.722
45	6.6	X = [10.0 - 6.6]/[10.0 - 5.5] =	0.755
90	5.5	X = [10.0 - 5.5]/[10.0 - 5.5] =	1.000
120	5.5	X = [10.0 - 5.5]/[10.0 - 5.5] =	1.000
180	5.5	X = [10.0 - 5.5]/[10.0 - 5.5] =	1.000
300	5.5	X = [10.0 - 5.5]/[10.0 - 5.5] =	1.000

F(X) was determined by the following expression (Chen et al., 1993):

$F(X) = 1 - 3(1 - 0.000)^{2/3} + 2(1 - 0.000) = 0.000$
$F(X) = 1 - 3(1 - 0.222)^{2/3} + 2(1 - 0.222) = 0.016$
$F(X) = 1 - 3(1 - 0.333)^{2/3} + 2(1 - 0.333) = 0.047$
$F(X) = 1 - 3(1 - 0.444)^{2/3} + 2(1 - 0.444) = 0.087$
$F(X) = 1 - 3(1 - 0.444)^{2/3} + 2(1 - 0.444) = 0.087$
$F(X) = 1 - 3(1 - 0.444)^{2/3} + 2(1 - 0.444) = 0.087$
$F(X) = 1 - 3(1 - 0.555)^{2/3} + 2(1 - 0.555) = 0.146$
$F(X) = 1 - 3(1 - 0.722)^{2/3} + 2(1 - 0.722) = 0.284$
$F(X) = 1 - 3(1 - 0.755)^{2/3} + 2(1 - 0.755) = 0.321$
$F(X) = 1 - 3(1 - 1.000)^{2/3} + 2(1 - 1.000) = 1.000$
$F(X) = 1 - 3(1 - 1.000)^{2/3} + 2(1 - 1.000) = 1.000$
$F(X) = 1 - 3(1 - 1.000)^{2/3} + 2(1 - 1.000) = 1.000$
$F(X) = 1 - 3(1 - 1.000)^{2/3} + 2(1 - 1.000) = 1.000$

The average binding site density of PVA (C^0) was determined from Equation (8):

$$C^0 = [C_0 - C_\infty] \left[\frac{volume\ of\ reactor}{volume\ of\ spheres}\right]$$

C^0 = [10 - 5.5 mg/L][500 mLs]/[70 mLs] = 32.1 mg/L

The integration of $\int$ Cdt was evaluated by the trapezoid rule as follows (Lewandowski and Roe, 1994):

t (min)	C (mg/L)	$\int$ Cdt (mg/L· min)		
0	10		=	0
2	9	$\frac{2 - 0}{(2)(1)}$ [10 + 9]	=	19
4	8.5	$\frac{4 - 2}{(2)(1)}$ [9 + 8.5] + 19	=	36.5
6	8	$\frac{6 - 4}{(2)(1)}$ [8.5 + 8.0] + 36.5	=	53.0
8	8	$\frac{8 - 6}{(2)(1)}$ [8.0 + 8.0] + 53.0	=	69.0
15	8	$\frac{15 - 8}{(2)(1)}$ [8.0 + 8.0] + 69.0	=	125.0
20	7.5	$\frac{20 - 15}{(2)(1)}$ [8.0 + 7.5] + 125.0	=	164.0
30	6.75	$\frac{30 - 20}{(2)(1)}$ [7.5 + 6.75] + 163.75	=	235.0

45	6.6	$\frac{45 - 30}{(2)(1)}$ [6.75 + 6.6] + 235	=	335.1
90	5.5	$\frac{90 - 45}{(2)(1)}$ [6.6 + 5.5] + 335.12	=	607.4
120	5.5	$\frac{120 - 90}{(2)(1)}$ [5.5 + 5.5] + 607.37	=	772.4
180	5.5	$\frac{180 - 120}{(2)(1)}$ [5.5 + 5.5] + 772.37	=	1102.4
300	5.5	$\frac{300 - 180}{(2)(1)}$ [5.5 + 5.5] + 1102.37	=	1762.4

Diffusivity (D) was determined from the following equation (Chen et al., 1993):

$$D = \frac{[Slope]C^0 R^2}{6}$$

$$D = \frac{(0.9625 \times 10^{-3} \text{ L/mg·min})(32.1 \text{ mg/L})(0.19 \text{ cm})^2(1 \text{ min}/60 \text{ sec})}{6}$$

$$= 3.1 \times 10^{-6} \text{ cm}^2/\text{s}$$

VITA

Patty S. Thompson

Candidate for the Degree of

Master of Science

Thesis: ANALYSIS OF PERMEABLE BARRIER TECHNOLOGY AS AN IN SITU GROUNDWATER REMEDIATION TOOL USING POLYVINYL ALCOHOL IMMOBILIZED CELLS

Major Field: Environmental Engineering

Biographical:

Personal Data: Born in Quincy, Illinois, July 23, 1952, the daughter of Ruby and David K. Knox.

Education: Graduated from Carney High School, Carney, Oklahoma in May 1970; received Bachelor of Science degree in Clothing, Textiles, and Merchandising and a Bachelor of Science degree in Geology from Oklahoma State University, Stillwater, Oklahoma in May 1974 and May 1988, respectively. Completed the requirements for the Master of Science degree with a major in Environmental Engineering at Oklahoma State University in July 1996.

Professional Experience: Geotechnician, Nicholas Exploration, Oklahoma City, Oklahoma, 1978 to 1981; Realty Director and Environmental Coordinator, Iowa Tribe of Oklahoma, Perkins, Oklahoma, 1989 to 1993; and, Realty Consultant, U.S. Dept. of the Interior, Bureau of Indian Affairs, Shawnee, Oklahoma, and Altex Resources, Inc., Tulsa, Oklahoma, 1993 to present.